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INTRODUCTION TO  
ANIMAL PARASITOLOGY



INTRODUCTION TO  
ANIMAL  
PARASITOLOGY

by

J. D. SMYTH

M.A., Sc.D.

*Professor of Zoology, Australian National University, Canberra.  
Formerly Professor of Experimental Biology, Trinity College, University of Dublin*

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*This book is affectionately dedicated to my  
students, whose interest and curiosity  
largely stimulated its production*



## PREFACE

This book is an attempt to provide a text introducing students to the study of animal parasites. In many laboratories, parasitology is still taught largely from stained sections, smears and whole mounts, with a sprinkling of living material here and there. This mode of teaching is understandable, for probably in no biological subject is a supply of fresh material so difficult to maintain.

Most of the major fields of parasitological research have been promoted on account of their economic or medical importance and this outlook has dominated the subject from the time when it was first realised that certain diseases were parasitic in origin. The study of a parasite as an organism living a specialised existence in a specialised environment has been, until recently, largely neglected, although it is encouraging to see this approach being more widely adopted in teaching and research laboratories throughout the world. It is now recognised that parasitic organisms in similar environments face similar kinds of problems and that it is from a study of these problems that the principles underlying the phenomenon of parasitism are likely to emerge. This is true whether the host be a man or a mouse, and the parasite the deadly *Plasmodium falciparum* or the harmless *Trypanosoma lewisi*. It is significant that many of the major parasitological discoveries were made on species non-parasitic in man. Ross's supreme discovery of the mosquito transmission of malaria, for example, was made on a species of *Plasmodium* in birds.

Throughout this text, then, special attention has been paid to those parasites which are available for examination and experiment in a *living* state, especially those from hosts which commonly are maintained, or could be maintained, in biological laboratories, and which are frequently studied in zoology syllabuses. Organisms such as these, although widely used for experimental studies, are given bare mention in most parasitological texts.

An effort has been made throughout to emphasise the physiological aspects of parasitism and in particular to analyse the factors controlling the growth, maturation and reproduction of parasites in relation to the physiology of their host and the properties of the environment.

Most of the better-known parasites of man are also considered, but no attempt has been made to cover the whole field of human or animal parasitology. Morphological descriptions in general have been kept brief, systematics treated broadly, and the pathological effects of parasites on their hosts have only been considered where they are related to some particular point of physiological interest. The more unusual parasites such as parasitic Mollusca or Crustacea have been omitted, as such forms are not sufficiently common to fall within the scope of this book. Thus the treatment is confined to those groups—protozoans, platyhelminths, nematodes and acanthocephalans—which have been most successful in invading the tissues or body fluids of animals. Arthropod vectors and so-called ectoparasites such as fleas or lice are also not covered here, since the morphology and biology of these groups have been adequately treated in a number of excellent text-books of medical or veterinary entomology.

Special attention has been paid to presenting the life cycles of parasites in detail, relating these, where possible, to the properties of the environment. When a life cycle is presented in diagrammatic form, in this way, the result is frequently complex, but it has been found that the preparation of such diagrams helps to focus attention on some overlooked aspects of the life cycle.

Prominence has also been given to two new and rapidly developing fields of parasitology, namely, immunity to animal parasites and the development of techniques for the cultivation of parasites *in vitro*. Within the past few years remarkable advances have been made in both these fields, and these open up exciting possibilities for the future.

Biological research is now being carried out on such a scale throughout the world that a text in most specialised fields is out of date almost as soon as it is published. This is certainly true in parasitology and the constant flow of reprints reaching the author each week serves to emphasise this fact. Many of the ideas set down here will require reconsideration in the light of further work, and this thought should be constant before the student, as he turns over the pages of this book.

Anyone who writes a modern scientific book must lean heavily on colleagues for help and advice. The writing of this book has been no exception, and it is a pleasure to thank those who have read and commented critically on substantial portions of the manuscript—Dr. Ann Bishop, Dr. W. H. Krull, Dr. C. A. Hopkins, Dr. Elspeth McConnachie, Dr. W. L. Nicholas, Dr. Gwendolen Rees, Professor J. Sprent and Dr. P. Tate. I am grateful to them for giving up valuable time in busy scientific careers in order to carry out this task. The assistance they have given in drawing attention to errors of fact or inconsistencies has been of inestimable value. Needless to say they



have not always agreed with my heterodox approach to certain aspects of parasitism, and in some cases I have modified my views as a result of their comments; in others, I have maintained independence! Such differences of opinion are, of course, the essence of scientific work.

I am also indebted to Dr. N. Kent for providing the original electron-microscope photographs of the cestode cuticle on which Fig. 89 is based; to Miss J. B. Williams for allowing me to use her unpublished figures of *Polystoma integerrimum* (Figs. 44, 45); to Dr. D. Howie for the unpublished figure of *Bucephalopsis gracilescens* (Fig. 60); to Mr. R. B. Rennison for the unpublished figure of *Diclidophora merlangi* (Fig. 47); to Dr. Mal Ferguson for providing a translation of some Russian literature; and to Dr. P. Silverman for the use of an unpublished MS.

Some illustrations are redrawn from original sources, and acknowledgments are made to the author by mentioning his name in the legend. As a matter of courtesy, the author's permission has been obtained, where possible, but a number of authors have been untraceable and in these cases their permission has been taken for granted. To the following publishers, I am indebted for permission to redraw illustrations from their publications:

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The great majority of illustrations have been drawn by Mr. Michael Riley, and I am grateful for the skill and imagination with which he so boldly interpreted my ideas. My thanks are also due to Mrs. N. Runham, Miss W. Heatley and Mrs. H. Taylor, who each contributed several illustrations.

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## CHAPTER I

# PARASITISM

### I.1 Animal Associations

The majority of animals live independently in their natural habitats, seeking their own food materials and utilising free water and oxygen for their metabolic processes. Between some animals, however, a variety of patterns of association has developed, and these may be broadly divided into two groups: homospecific associations—those between individuals of the same species; and heterospecific associations—those between individuals of different species.

Individuals of the same species may form loosely united communities, such as herds of cattle or flocks of sheep, while others, such as some species of ants or bees, may form elaborately organised communities in which individual members often exhibit considerable division of labour or specialisation.

Heterospecific associations are in general much more complex and a number of terms have been developed to describe them. Like many terms used in biology, these are essentially operational words which are definable only within broad limits. They do, nevertheless, serve a useful role in enabling us to file data into convenient, though not water-tight, compartments. Terms such as *commensalism*, *phoresis*, *symbiosis*, *mutualism* and *parasitism* have been widely used in the biological literature for various types of heterospecific association, and their definition has been discussed by a number of workers, especially Lapage (1951), Cameron (1956), Baer (1952) and Caullery (1952). These terms were developed in a period when little data on the possible physiological basis of such associations were available. Within recent years, the situation has changed somewhat, and although information is still meagre, the general increase in knowledge of animal physiology and biochemistry enables these associations to be considered on a broader basis than was hitherto possible.

*Commensalism* and *phoresis* represent only loose associations made on a basis of shelter, defence or food-obtaining mechanisms. *Symbiosis*, *mutualism* and *parasitism*, on the other hand, are intimate associations in which the metabolism of an individual of

one species is dependent to some degree on permanent association with an individual of another species. The concept developed here is that it is this metabolic dependence of one species on another which separates these intimate kinds of association very markedly from those of the looser kind. As explained further on p. 3, mutualism and symbiosis are here considered as special cases of parasitism in which mutual metabolic dependence occurs.

Although this text is concerned with the phenomenon of parasitism in particular, it is worth while to consider briefly the other types of heterospecific associations in order to place parasitism in its true perspective.

### 1.2 Commensalism

The term literally means 'eating at the same table', and there are a number of often-quoted classical examples of this type of loose association between animals of different species. One of the best known is that between certain species of hermit crabs and sea-anemones, in which the anemone lives on the shell sheltering a hermit crab. The sea-anemone benefits directly, having access to the food caught and scattered or unwanted by the crab, whereas the crab benefits by the presence of the sea-anemone which assists in warding off undesirable predators. In many cases, although this type of association is beneficial to one or both organisms, it is not usually obligatory for their existence. An exception is the association between the hermit crab, *Eupagurus prideauxi*, and the anemone, *Adamsia palliata*, in which neither of the partners is able to survive alone. The crab crawls into a shell which is too small for itself and uses the pedal disc of the anemone as cover for the unprotected portion of its body.

Other examples of commensalism are of an even less intimate nature. For example, the association between the oxpicker bird and various African mammals. The birds feed on the lice and ticks of mammals such as rhinoceroses and serve to warn them of approaching enemies by displaying their own independent reactions.

Commensalism may thus be considered a type of loose association in which two animals of different species live together *without either being metabolically dependent on the other*, although one or both organisms may receive some benefit from the association. It is important to stress the absence of *metabolic* dependence in this type of association, for it is the absence of this feature, in particular, which separates a commensal sharply from a parasite. The association is not intimate—since the tissues of the commensals are not in organic contact—nor need it be permanent.

### 1.3 Phoresis

This term is used for a particular type of association in which one organism merely provides shelter, support or transport for another organism of a different species. The term *phoresis*, however, is not widely used in biological terminology and will be only briefly mentioned here.

The classical example is that of fishes belonging to the genus *Fierasfier* which live within the respiratory trees of holothurians, or occasionally starfish. These fish are relatively helpless and are readily attacked and devoured by other species. The holothurians appear to be undisturbed by the presence of the fish.

In phoresis, as in *commensalism*, then, there is no metabolic dependence of either of the associates on the other. This type of phenomenon could clearly represent a stage similar to that in the early evolution of parasitism, since chance contact followed by the use of one species as shelter by the other is likely to have been the first step in an association leading to the parasitic way of life.

### 1.4 Parasitism, Mutualism and Symbiosis

In the type of association which forms the subject matter of this book, contact between the individuals of two different species differs markedly from that already described in that it is intimate and continuous. Many parasites have free-living stages in their life cycles and only during the periods when they make contact with their hosts can they actually be considered to lead a parasitic existence.

Of all the types of animal associations, perhaps parasitism has been, in the past, the most difficult to define. This has been largely due to the failure to recognise that the term has only a relative meaning but also to the insistence, by most authors, that a parasite must necessarily be harmful to its host animal. In the writer's opinion, this emphasis on the harmful effects of a parasitic association has bedevilled a rational approach to considering the phenomenon, more than any other single factor.

Studies on the metabolism and biochemistry of so-called parasites have led to the development of the concept that when the phenomenon is considered in the light of the metabolic dependence of one species on another, many of the difficulties inherent in the older definitions disappear (Smyth, 1961). Moreover, the relationship between parasitism, mutualism and symbiosis becomes more rational.

On this view then, to be classified as a parasite an organism must not only be in continuous intimate association with an individual of a different species, but it must also be metabolically dependent on it to some degree. Parasitism is seen thus as a relative phenomenon, and it is possible to draw up a list of parasitic species which show an increasing degree of metabolic dependence on their hosts. At one end of this hypothetical scale (Fig. 1) is zero dependence, i.e. a free-living organism; at the other end is 100 per cent dependence or total parasitism. In between these two extremes are a range of organisms which satisfy their metabolic requirements to a varying extent at the expense of the host.



We must make clear here what we mean by 'metabolic dependence'. Although at first sight it would appear that most parasites are only dependent on their hosts for food materials, a closer examination shows that the situation is more complex than this. Examples can be given of parasites which are dependent on their hosts for one or more of the following: (a) developmental stimuli; (b) nutritional materials; (c) digestive enzymes; (d) control of maturation.

The plerocercoid larva of the cestode *Schistocephalus solidus* presents a classical case of the provision of a developmental stimulus by the host. This species (p. 248) is progenetic and the genitalia reach an advanced stage of organogeny in the larval condition

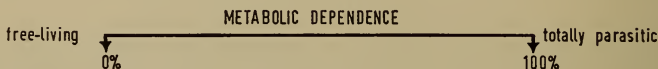


FIG. 1. Diagram showing the relative concept of parasitism based on the degree of metabolic dependence. A free-living organism shows zero dependence; a cestode shows virtually 100 per cent dependence. All degrees between these two extremes are encountered.

while parasitic in fish. On ingestion of an infected fish by a bird, the larva reaches sexual maturity and produces eggs within thirty-six hours (Fig. 105). It can be readily demonstrated experimentally (p. 421) that the stimulus for maturation of this larva is the provision of heat-energy by the host, and that the same effect can be produced by culturing the larva *in vitro* in a non-nutrient medium, provided suitable physico-chemical conditions are maintained. Here then, theoretically, is a condition of 'energy parasitism'—an association in which the parasite is dependent on the host for the heat-energy necessary for it to reach maturity. This is an exceptional case, but it must be borne in mind that parasites of homoiothermic animals are receiving a heat-transfer from their hosts in addition to any other benefits.

Dependence on the host for nutritional materials is undoubtedly the commonest form of metabolic dependence. A range of levels occurs and parasites may be dependent for nutritional supplies on (a) the food of the host either before or after digestion; (b) the tissues of the host; or (c) secretions of the host. In most parasites, all the nutritional requirements are satisfied by the host but some parasites may obtain additional materials from extraneous sources. Consider, for example, the case of a monogenetic trematode (p. 47) parasitic on the gills of a fish. This parasite feeds on the blood vessels of the gills, but is able to digest the engorged blood by means of its own enzymes and utilise the breakdown products obtained. Due to the close contact with sea water, however, it almost certainly has access to extraneous oxygen by diffusion. Thus in the synthesis of



the parasite tissue, some of the oxygen molecules utilised for synthetic or energy purposes will have been derived from non-host sources. Nutritional dependence on the host can thus not be said to be complete.

In contrast, species of blood flukes, e.g. *Schistosoma haematobium* (p. 185) in the mesenteric veins of man are 100 per cent dependent on the host for all nutritional supplies including oxygen. Thus, in the synthesis of parasite tissue the percentage of atoms of host origin which are metabolically involved could be used as a means of estimating the nutritional dependence on the host. Such an estimate would go a long way towards providing an unequivocal measurable evaluation of the degree of parasitism by a particular species. This is, of course, a *theoretical* approach and, although possible in practice, technical difficulties make it impractical for general use. A quantitative approach along these lines does away with the difficulties which arise when subjective terms such as 'partial' or 'total' parasitism are used; it does not, however, measure some of the other factors in parasitism yet to be discussed.

Dependence of a parasite on the host for its food materials is of little value unless it can utilise the food thus obtained. There is increasing evidence that the majority of trematodes and nematodes possess the digestive enzymes necessary to hydrolyse complex molecules. Many parasites, like the monogenetic trematode quoted above, can digest blood and tissue. The larva of *Diplostomum phoxini* (p. 83), for example, can digest an egg-albumen mixture *in vitro* and utilise the breakdown products for the processes of growth and differentiation. Such organisms are therefore not dependent on the host for digestive enzymes. On the other hand, animals such as tapeworms or acanthocephaleans—which lack a gut—can only utilise molecules of a size capable of absorption through their cuticle and are entirely dependent on the host's ability to break down carbohydrates, fats and proteins enzymatically. In these cases then, dependence on the host has proceeded a stage further than in nematodes and trematodes.

Finally, there are a small number of species of parasites which are dependent on the host for the control of their maturation processes. Such examples represent a remarkable stage in the evolution of parasitism, whereby an endocrine system developed to stimulate metabolic processes in one animal is utilised by another of a different species. This results in the synchronisation of the reproductive phases of host and parasite, an effect which has considerable survival value for the parasite. For example, the blood protozoon *Leucocytozoon* (p. 108) undergoes a multiplication phase resulting in a rapid increase in the number of its gamete-producing cells (gametocytes) only during the breeding season of its duck host—a time which corresponds to the natural occurrence of its vectors (black flies). Although it has not yet been shown that this phenomenon

is directly due to the hormonal mechanism of the reproductive cycle, it is, at least, likely to be closely associated with it. A similar example is found in the flagellate protozoans, symbiotic in the wood-eating roach *Cryptocercus*, which undergo sexual cycles only during the moulting period of the host (Cleveland, 1949-59). This phenomenon is thought to be due to the influence of the neurosecretory cells exerted through the prothoracic-gland hormones, although this has not been definitely established (Cleveland and Nutting, 1955).

An even more striking example is presented by certain parasites of the frog—the ciliates *Opalina ranarum* (p. 114) and *Nyctotherus condiformis* (p. 121), and the monogenetic trematode, *Polystoma integerrimum* (p. 129). The life cycles of these organisms are beautifully synchronised with that of the amphibian host and involve release of cysts or eggs at a time when the frog is breeding in water, i.e. when potential tadpole hosts are assured. It has been shown experimentally that maturation of the parasites is related to the levels of sex-hormones in the host and may be induced experimentally by injecting suitable hormones into the host (Mofty and Smyth, 1960).

It is clear from the above account that the metabolic dependence of a parasite on its host is a complex matter involving a number of factors. This concept of parasitism does not take into account the harmfulness or otherwise of the association between host and parasite. In some cases the association between host and parasite is such that the host is damaged in some way, perhaps by physical injury, by loss of essential nutrients, or by the toxic effects of excretions or secretions of the parasite. In other instances no harm results, and in still others metabolites released by the parasite may be beneficial to the host. Whether an organism is harmful or not is irrelevant to the metabolic concept of parasitism as outlined above.

An association in which both associates benefit has long been referred to as *mutualism* by some authors and *symbiosis* by others. The literature on the definition of these words is confused. Mutualism is derived from the Latin *mutuus* (= exchanged), whereas symbiosis comes from the Greek *sympion* (= to live together). The term *symbiosis* could thus broadly be used to include all the different kinds of relationship which exist in nature. By usage, however, it has come to be restricted to associations of a special kind in which the participating species are dependent on each other for existence. This is clearly only a convention, but general agreement to restrict the use of the word in this way would do much to clarify the terminology. In cases of mutualism, on the other hand, the association is not obligatory to existence. On the metabolic view put forward above, both mutualism and symbiosis are merely recognised as special cases of parasitism in which some metabolic by-products of the parasite are of value to the host.

There are several well-known examples of the phenomenon. The association between wood-eating termites and hyperflagellates in their intestine is of the mutualistic type of parasitism. The termites are entirely dependent on the flagellates for certain nutritional requirements, notably the supply of nitrogen and carbohydrates obtained by the breakdown of wood. The flagellates are similarly dependent on the host for nutriment and the physical environment in which they live. The dependence of the host on the flagellates may be readily demonstrated by raising the termites to a temperature which is lethal to the protozoans, thus defaunating them. Under such conditions, the termites fail to survive, as they lack the enzyme systems necessary to digest a wood diet.

The association between intestinal ciliates and their ruminant hosts (discussed on p. 122) is of a similar nature. Experimental work has shown clearly that several genera of ruminant protozoans produce the enzymes cellulase and cellobiase, thus enabling them to split cellulose and utilise the breakdown products for their metabolism. The rate of fission of these ciliates is extremely rapid, and they soon die. On disintegration, they suffice to provide the host with about one-fifth of its total nitrogen requirements.

An example of an association which would be considered to be mutualistic is that between the coelenterate *Hydra viridis* and the alga *Zoochlorella* which lives within its endodermal cells. The alga produces oxygen which *Hydra* utilises and *Zoochlorella* makes use of the nitrogenous waste products of *Hydra* for its synthetic processes. It is possible that a mutualistic association exists in many cases of parasitism, but sufficient physiological studies have not been made to reveal their existence.

Once the relative connotation of the term 'parasite' is accepted, parasites can be classified in other ways according to their life cycles, position on the host, or various other features. It is common practice, for example, to speak of *ectoparasites* and *endoparasites*. *Ectoparasites* are organisms (e.g. fleas, lice, ticks) which live on the outside of their hosts, usually attached to the skin, feathers, hair, gills, etc.; such forms can never lead a completely parasitic existence, but utilise oxygen from outside the host. Many maintain only periodic contacts with their hosts and, according to the definition given earlier, cannot be considered parasites but essentially special kinds of predators. *Endoparasites* are parasites living within their hosts, in the gut, body cavity, lungs or other tissues; such forms nearly always live a completely parasitic existence. Certain parasites fall into both these groupings. The itch mite (*Sarcoptes scabiei*), for example, burrows in tunnels in the skin and could satisfy the criteria of either an ectoparasite or an endoparasite. Again then, these terms cannot always be accurately defined, but they are convenient general terms. Parasitologists also speak of *facultative* parasites, organisms which can live either a parasitic or non-parasitic existence, and *obligate* parasites which

are obliged to live a parasitic existence and are incapable of surviving in a naturally occurring free-living environment. In the latter definition, the emphasis must be on a 'naturally occurring' environment for many 'obligate' parasites can now be cultured in artificial environments of a complex nature (pp. 396-434).

To invade the body of another species of animal, and to live and multiply in or on it, could not have been achieved without considerable morphological and physiological adaptation on behalf of the parasite. Some of these physiological adaptations have been discussed here and are covered more fully in the text. It is not intended, however, to discuss the morphological adaptations of parasites to their way of life. These have been discussed in detail by numerous authors, especially Baer (1952) and Caullery (1952), and a considerable literature exists on this aspect of parasitism. It is unfortunate that we know so relatively little concerning the physiological adaptations of parasites, since a coherent picture of the processes underlying parasitism can only emerge when both morphological and metabolic aspects of the phenomenon are fully taken into account.

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## CHAPTER II

# HABITATS AND ENVIRONMENTS OF PARASITES

One feature of the evolution of metazoan organisms is the increasing complexity of their alimentary, respiratory and circulatory systems. The development of such systems was, of course, advantageous to these evolving organisms and it resulted in a more efficient metabolism, but it was not without some inherent disadvantages. Each new organ system evolved—especially those containing cavities or surfaces, presented a habitat for potential parasites. These cavity-containing organs appeared especially in vertebrates; every part of the vertebrate body capable of supporting parasitic life has been invaded. The favoured habitats in vertebrates are (*a*) the alimentary canal and its associated glands; (*b*) the blood stream; (*c*) the respiratory system; and (*d*) the coelom, in that order. The nervous system and its derivations, the excretory system and the reproductive system have also been invaded, but less commonly so than the larger tubular systems. In the case of invertebrates, possible habitats are fewer, and only the alimentary canal, its associated glands and the haemocoel have been invaded to any extent.

This chapter is concerned with the nature of the environmental conditions provided by some of these parasite habitats, only the more important being considered. Detailed analysis of all possible habitats would involve consideration of virtually every organ and tissue in the vertebrate body as well as many in invertebrates; such an analysis is beyond the scope of this book.

In the case of organisms with complex heteroxenous life cycles (i.e. those involving more than one host), the question of the part played by environmental conditions in controlling the development of the parasite is one of considerable interest, and one scarcely touched on by modern parasitological research. The physico-chemical conditions of a habitat— $pH$ ,  $pCO_2$ , oxygen tension, oxidation-reduction potential, temperature, viscosity, osmotic pressure—are of major importance, as are the nature, quantity



and availability of food materials. For example, egg-production in certain trematodes is believed to be limited by the degree to which the environmental food supplies can satisfy the enormous synthetic demands of the egg-producing stages, with the result that, in general, mature trematodes are limited in their distribution to environments with high nutritional 'levels', (see p. 22). The ecological pattern here is thus not very different from that of free-living forms.

Many parasites pass through a number of habitats, differing widely in their properties. *Cryptocotyle lingua*, an intestinal trematode of a bird (p. 179), for example, passes through water, mollusc tissue (first intermediate host) and fish tissue (second intermediate host) before reaching its final location in the bird intestine. The environmental conditions and food supply in these habitats may vary within wide limits.

Considerations of this kind have led to the concept of the physiological life cycle, which concerns itself with the physico-chemical characteristics of the habitat of the parasite at each stage. When this type of information is integrated with the morphological considerations of the life cycle, the combined result is a more informative and interesting overall picture of the biology and behaviour of the organism; where possible life cycles have been treated on this basis throughout this book.

## 2.1 The Vertebrate Alimentary Canal

### 2.11 General Properties

The vertebrate gut may be regarded as one of the most hazardous habitats for a would-be parasite. It is dark; it is undergoing regular physiological changes relative to the feeding habits of the host; it contains a battery of protein, fat and carbohydrate-splitting enzymes; its pH may range from 1.5 to 8.4; it is almost oxygen-free, and it often undergoes violent movements. Each of these features, to a greater or lesser degree, presents a problem to an organism attempting to live and reproduce in it.

The physical and chemical properties of the gut are discussed separately below, and the special environmental conditions pertaining to each part are dealt with later. Most of the information is based on the mammalian alimentary canal; this has been reviewed by Hobson (1948) and Read (1950, 1955). With the exception of that of insects, the intestine of invertebrates has been little studied.

*Oxygen tension.* This question has claimed some attention on account of the possible significance in the aerobic or anaerobic metabolism of parasites. Although abundant air may be available in the mouth and oesophagus and some in the stomach, the bulk of evidence points to the almost complete absence of oxygen in the remainder of the alimentary canal. The available details are summarised in Table 1. The figures for the

TABLE I  
OXYGEN TENSIONS IN SOME HABITATS OF PARASITES;  
FIGURES GIVEN IN mm Hg  
(data from von Brand, 1952)

Habitat	Host species	O <sub>2</sub> tension	Habitat	Host species	O <sub>2</sub> tension
Skin	man	50-100	Bile	cattle, sheep, dog	0-30
Subcutaneous tissue	man, rat, pig, etc.	20-43	Abomasum (near mucosa)	sheep	4-13
Arterial blood	man, dog, fish	70-100	Rumen (gases)	cattle, sheep, goat	0-2
Venous blood (heart)	man, horse, duck	37-40	Stomach (gases)	man	0-70
Venous blood (portal vein)	dog, cat, etc.	49-66	Small intestine (near mucosa)	sheep, rat	4-30
Peritoneal cavity	rabbit, rat, cat	28-40	Small intestine (gases)	horse, cattle, dog	0-6
Pleural cavity	man, monkey	12-39	Small intestine (gases)	pig	8-65
Urine	man	14-60	Large intestine (gases)	horse, cattle, rabbit	0-5

pig are relatively high, a result possibly attributable to the swallowing of air during slaughter.

Direct analysis of the gaseous content of the lumen of the intestine may not reflect accurately the oxygen tension in the immediate vicinity of the parasite, as other factors must be taken into consideration. The microfloral content, for example, will have a profound effect. A striking case of the influence of bacteria is shown by *Trichomonas buccalis*—a flagellate parasite of the mouth. The mouth is *a priori* an aerobic habitat, and yet *T. buccalis* like other trichomonads (p. 49) has been shown to be an obligate anaerobe. This is explicable only by assuming that the microflora of the mouth depletes the mucous environment of virtually all its oxygen.

Indirect experimental evidence also supports the view that the vertebrate gut is an anaerobic environment. Many trematodes and pseudophyllidean cestodes produce eggs whose shell is a quinone-tanned protein and characteristically turns brown on exposure to air. The vitelline glands which secrete the bulk of this shell material likewise go brown in organisms exposed to air *in vitro*. Yet intestinal trematodes removed fresh from the gut are colourless, indicating an oxygen tension sufficiently low to inhibit 'tanning' of the vitellaria.

It has been stressed that the oxygen tension in the mouth cavity does not necessarily indicate the actual conditions under which organisms in contact with the mucosa are living. It is becoming increasingly evident that the oxygen tension must be measured

as near the true site of the parasite as possible. By the use of micro-electrodes, for example, it has been found (Rogers, 1949) that the thin layer of liquid in contact with the mucosa of the small intestine gives a figure *three times higher* than in the bulk of the intestinal content. Thus figures for the  $O_2$  tension of habitats of parasites should be accepted with a degree of caution and subject to confirmation by methods capable of precise localisation.

All that can generally be concluded, therefore, is that the lumen of the gut is essentially anaerobic, with probably an increasing oxygen gradient in the mucous film covering the mucosa lining.

Some workers, notably Read (1950), consider that the physico-chemical conditions adjacent to the mucosa are sufficiently different from the centre of the lumen to justify the use of a special term, the *paramucosal lumen* for this region; conditions there may be nearer those of the intercellular spaces of the host than the intestinal lumen.

*Other gases.* Nitrogen, hydrogen, methane and carbon dioxide may also be present in varying quantities. The intestinal contents are usually saturated in carbon dioxide, and this gas may play an important part in the regulation of the intracellular hydrogen ion concentration of the parasites. It may also play an important role in helminth life cycles. For example, Rogers (1958) has shown that  $CO_2$  at low redox potentials is concerned in stimulating the infective eggs of the nematode *Ascaris lumbricoides* to release a hatching fluid containing the enzymes (an esterase, a chitinase and possibly a protease) concerned in hatching.

*Oxidation-reduction potential.* This characteristic has been little investigated. The following Eh values for the different regions have been given: large intestine and caecum, — 195 to — 200 mV; small intestine, — 100 mV; stomach, + 150 mV.

*Hydrogen-ion concentration.* The hydrogen-ion concentration alters sharply from region to region. In mammals, on which most work has been carried out, the pH of the mouth is usually about 6·7 but may show a range of 5·6–7·6. In the stomach, strongly acid conditions prevail but the degree of acidity varies remarkably with the condition of the animal. Typical figures for the pH of gastric juice are: man, 1·49–8·38; cattle, 2·0–4·1; sheep, 1·05–3·6; mouse, 3·26–6·24 (Haiba, 1954). In the duodenum, the pH in most mammals is just on the acid side, about 6·7, but shows a range of 5·8–7·6 (man); in the cat and goat the duodenal secretion is more alkaline with a pH of 8·2–8·9. That the duodenum is usually an acid habitat is not generally appreciated; it is commonly held to be alkaline or even strongly alkaline. This misconception is based on the values for the succus entericus, bile and pancreatic juice, which, although showing a range of figures, tend to be weakly alkaline. The gastric contents shed from the stomach into



the duodenum are strongly acid, and the intestinal secretions are seldom sufficiently alkaline to neutralise them completely. In the region of the large intestine, the pH rises to about 8.4.

Protozoan cysts, helminth eggs, or larval worms thus have to run the gauntlet of severe pH changes in passing from mouth to intestine, and these changes may play an important role in the release of larvae from cysts or eggs, or in the excystment of protozoans.

*Osmotic pressure.* Variations in osmotic pressure of the gut contents during feeding or starvation have not received much attention. The few analyses which have been made show that in man (Table 3) the osmotic pressure tends to be hypertonic to the blood ( $\Delta = -0.56^\circ \text{C.}$ ) on passing from the stomach ( $\Delta = -0.3$  to  $-0.8^\circ \text{C.}$ ) to the duodenum ( $\Delta = -0.63^\circ \text{C.}$ ).

*Bile.* In view of the popularity of the bile duct as a parasite habitat, particularly for trematodes, the composition and properties of bile are of special interest. Bile contains inorganic salts, mucin, pigments and lipids, nucleo-protein, cholesterol, carbon dioxide, ammonia, urea, and purine derivatives, but the proportion of these, as well as the nature of the pigments, varies considerably with the animal.

Some properties of animal biles are shown in Table 2. The oxygen tension of bile is low (0.30 mm. Hg) so that like the gut, the bile duct may be considered essentially an anaerobic habitat. It may be noted that the pH range of bile from the liver (hepatic bile) is generally higher than that stored in the gall bladder and later

TABLE 2  
COMPARATIVE PROPERTIES OF BILE  
(mainly after Read, 1950)

Species	O <sub>2</sub> tension (mm Hg)	Daily secretion per kilo body wt. (ml)	pH		$\Delta^\circ \text{C.}$	Cholesterol content mg/%	Glucose mg/%
			Hepatic bile	Gall- bladder bile			
Man . . .	—	15	6.2-8.5	5.6-8.0	-0.56	—	—
Dog . . .	0.30	8-76	7.1-8.2	5.2-6.9	—	110-140	55-88
Cat . . .	—	14	—	5.3	—	—	—
Sheep . .	0.30	21-30	5.9-6.7	6.0-6.7	-0.59 to -0.6	—	—
Rabbit . .	—	74-219	—	6.4-6.7	—	100-120	20
Ox . . .	—	—	—	6.7-7.5	-0.53 to -0.6	30-70	—
Guinea pig	—	160-200	7.7-7.8	7.2-9.1	—	—	—
Rat . . .	—	40-60	—	—	—	—	—
Goose . .	—	6-18	—	—	—	—	—
Crow . .	—	13-110	—	—	—	—	—

secreted. Bile has considerable power of buffering which may account for the ability of large parasites, such as *Fasciola*, to survive in the confined habitat of the bile duct, in spite of secreting acidic metabolic waste products which might otherwise produce a high local pH. Most mammalian biles contain the salts of cholic acid and deoxycholic acid conjugated with taurine and glycine. Other characteristic acids are lithocholic acid, cheno-deoxycholic acid and hyodeoxycholic acid. Many other animals possess unique bile acids. The salts of the bile acids serve mainly to lower the surface tension and to promote the emulsification of food colloids and the intestinal absorption of lipids.

The inorganic constituents of bile have no unusual qualities, being present in quantities to be expected in a transudate of blood.

Of the other constituents, two deserve particular mention—cholesterol and glucose. The former may be of importance, for some invertebrates (e.g. *Trichomonas*, p. 49)

TABLE 3  
PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE DIGESTIVE  
FLUIDS OF MAN

(data from *Handbook of Biological Data*, 1956)

Constituent or property	Gastric juice	Bile (hepatic)	Bile (gall-bladder)	Pancreatic juice	Duodenal secretion
pH . . . . .	1.49–8.38	6.2–8.5	5.6–8.0	7–8	5.8–7.6
Δ°C. . . . .	–0.3 to –0.8	–0.56	–0.56	–0.63	?
Chloride total mEq/L . . . . .	78–159	75–110	15–30	60–80	64.2–110
Sodium mEq/L . . . . .	49	?	?	138	84.8–143
Potassium mEq/L . . . . .	11.6	?	4.9	4.1–5.6	1.0–11.0
Protein mg/100 ml. . . . .	330	275	315–540	190–340	?
Glucose mg/ml. . . . .	0.35–1.19	17–52	80	8.5–18.0	?
Solids, total mg/100 ml. . . . .	?	2,660	11,140	1,240–1,540	1,500

require cholesterol but are unable to synthesise it. The presence of glucose may also be significant, as a source of energy for some organisms.

Bile salts play a major role in stimulating larval cestodes to evaginate the scolex. Concentrations as low as 0.01 per cent produce some activity of the cysticercoid larvae of *Hymenolepis diminuta*, and concentrations of 0.3 per cent produce about 40 per cent evagination. When cysticercoids are treated with bile salts and trypsin, a synergistic effect is observed and some 90 per cent larvae excyst (Read, 1955). The stimulating effect of bile and bile + trypsin is evidence of a beautifully developed mechanism for ensuring that excystation occurs only in the duodenum or the later regions of the alimentary canal. This mechanism may operate in many helminth life cycles; the

hatching of the eggs of cyclophyllidean cestodes is probably the commonest example.

*Enzymes.* The properties and distribution of digestive enzymes are too well known to require much comment, but they are listed here for reference purposes (Table 4). During digestion, carbohydrates are broken down to monosaccharides, fat to glycerol and short-chain fatty acids, and proteins to amino acids. Parasites bathed in these released food materials will be in active competition for them with the cells of the mucosa, and

TABLE 4  
DIGESTIVE ENZYMES IN THE ALIMENTARY CANAL OF MAN

T = present in tissue. S = present in secretion.  
(data from *Handbook of Biological Data*, 1956)

Enzyme	Salivary glands	Oesophagus	Stomach	Pancreas	Small intestine	Caecum and colon
Amylase . . . .	S	—	—	S	T, S	—
Enterokinase . . . .	—	—	—	—	S	—
Erepsin . . . .	S	—	—	T, S	T, S	—
Sucrase . . . .	—	—	—	—	S	—
Lipase . . . .	T, S	T	T, S	T, S	S	—
Maltase . . . .	—	—	—	S	—	—
Pepsin . . . .	—	—	T, S	—	—	—
Phosphatase . . . .	S	—	T	—	T	T
Rennin (chymosin) . . . .	—	—	S	—	—	—
Trypsin . . . .	—	—	—	T	—	—
Urease . . . .	—	—	T	—	—	—

in the case of certain growth factors (e.g. vitamin B<sub>12</sub> which is absorbed by the cestode *Diphyllobothrium latum*) at least, absorption by the parasite may produce levels seriously below those required by the host.

It is not, however, only the soluble food materials which are absorbed by intestinal parasites, for the alimentary canal of the few trematodes and nematodes which have been investigated have been shown to possess a wide range of digestive enzymes. This indicates that complex food materials can also be digested.

## 2.12 General Environmental Conditions in Different Regions of the Alimentary Canal

The mouth, oesophagus and stomach rarely act as habitats for parasites. In fishes and amphibians, trematodes and copepods may occur in the mouth. In man, the flagellate *Trichomonas buccalis* occurs in the same site. The stomach of cattle may contain the nematode *Haemonchus contortus* and the larvae of bot flies of the genus *Gastrophilus*.

By far the most popular sites for intestinal parasites are the duodenum, the ileum, the caecum and the large intestine. It is worth while considering in a little more detail the special structural details of these regions, especially as they affect the biology of parasites.

*Duodenum.* This region is characterised by possessing special glands, Brunner's glands, which in many species are continuous with the pyloric glands. They are composed of ramifying tubules which empty by many ducts into the crypts of Lieberkühn in the overlying mucosa. These crypts make ideally sheltered environments for some protozoa. Most duodenal juice is mucoid, but the degree of stickiness varies. In the rabbit, goat and sheep the juice is thick and more like egg-white, while that from the pig, cat and dog may be pulled out in tenacious threads.

It is difficult to visualise the exact conditions within the duodenum. Food is passed from the stomach in a partly digested condition having been attacked by salivary amylase and stomach pepsin. In the upper duodenum, as already noted, the pH rises sharply—probably rarely to alkalinity—and over the food are poured bile and pancreatic juice with their batteries of enzymes (Table 4). Protein breakdown continues, and proteoses, peptones, polypeptides and some amino acids are released; monosaccharides result from further breakdown of carbohydrates, and glycerol and fatty acids from the hydrolysis of fats.

This region then is rich in highly nutrient food materials, although the amount of monosaccharides available at any instant is probably small, for polysaccharides are only slowly broken down, and glucose especially is actively absorbed. It is not surprising then that the larger parasites should favour this region in which to grow. Radiological studies have shown that physically, like the stomach, it is a region of comparative calm, whereas further down the duodenum the food boli are torn and shredded, as one worker puts it, in a manner 'comparable with a mincing machine'. The food is propelled to and fro, turned over and broken up, a process of agitation termed 'fragmentation'. This process is not to be confused with peristalsis which is one in which gentle waves of contraction pass over the stomach, down the pylorus and along to the small intestine.

Fragmentation, while clearly making it difficult for parasites to maintain their position, by its very violence serves to bring new food materials into contact with intestinal parasites and also assists in the removal of waste products by increasing the rate of diffusion.

*Ileum.* Further down the small intestine, the availability of food material for intestinal parasites decreases due to the absorption of amino acids and carbohydrates through the

portal radicles, and of fat by the lymphatics. There is another possible source of nutriment available and this is the cells of the intestine itself. There seems no doubt that this region is one of considerable wear and tear, so that a constant sloughing off of cells takes place. These desquamated cells are rich in enzymes and probably contribute substantially to the enzymes available in the mixed intestinal juice (the *succus entericus*). An abundant bacterial fauna also provides a possible source of nutriment (Table 5). *Colon and Caecum.* The caecum is an especially favoured site for parasites, such as entamoebae, scavenging bacteria and food detritus. These are regions of comparative inactivity. The mucous membrane contains crypts, but no villi. Much mucus may be secreted. The chief function of the caecum and colon is to absorb water, at least in the carnivora, for which most information is available. In the herbivora, these regions are the site of considerable digestion by bacterial action, especially of cellulose. The presence of small quantities of enzymes (phosphatases) have been reported, but the main secretion in this region is mucus.

*Viscosity.* The mucosa of the vertebrate gut is covered with a film of mucus, even in the fasting animal, and its character varies somewhat in the different regions. In general, it has a viscid surface suitable for the adhesion of many types of parasites. In autopsy

TABLE 5  
BACTERIAL CONTENT OF THE HUMAN INTESTINE  
(data from Martini, Phear, Ruebner and Sherlock, 1957)

Site	Total No. of specimens	Coliform <sup>1</sup>	Str. faecalis	Transients <sup>2</sup>	Proteus, Ps. pyo., Bact. f. alk.	Anaerobes <sup>3</sup>
Duodenum . . .	7	0	1	4	0	1
Jejunum . . .	6	0	0	4	0	2
Ileum . . .	7	3	1	4	2	3

<sup>1</sup> Coliform organisms included: *Esch. coli*, *Esch. freundii*, *Klebsiella*, *Bact. cloacae*, *Paracolon bacille*.

<sup>2</sup> Transient organisms included: Streptococci (non-haemolytic), *Streptococcus viridans*, Neisseriae, Diphtheroids, Staphylococci (coagulase + ve), *Candida*, *Anitratum* group.

<sup>3</sup> Anaerobes included: Streptococci (anaerobic), *Lactobacille*, *Bacteroides*.

examination of the gut, a tapeworm, for example, is usually found flattened and stretched with its surface adhering closely to the mucosa. On removal it may contract perhaps to one-quarter of its relaxed length. This close adhesion to the intestinal mucosa is probably essential to cestodes for absorption of food materials and diffusion of waste materials, as well as being necessary to compress the strobila sufficiently to enable the cirrus in each proglottid to be bent into an adjacent proglottid, thereby permitting insemination and fertilisation to take place (see p. 232).



## 2.2 Blood

The chemical composition of human blood is shown in Table 6. Like the alimentary canal, the quantity of soluble food materials in the blood stream will vary with the feeding habits of the host. As a source of nutriment, its value must be considered in relation to the morphology or physiology of the organism utilising it. To a cestode, which lacks a gut and is dependent on small molecules of absorbable dimensions, such as amino acids or glucose (p. 277), it is a relatively poor medium compared with that provided by the duodenum. On the other hand, to a trematode such as *Schistosoma mansoni* (p. 185), which possesses both a gut and a well-developed digestive enzyme system, the quantity of protein in the plasma and blood cells represents a diet of a

TABLE 6  
CHEMICAL CHARACTERISTICS OF BLOOD AND SEROUS FLUIDS OF MAN

—values mg/100 ml, unless otherwise indicated  
(data from *Handbook of Biological Data*, 1956)

	Whole blood	Red blood cells*	Plasma	Serum	Pleural fluid	Peritoneal fluid	Cerebro- spinal fluid	Lymph
pH . . . . .	7.4	?	7.39	7.4	?	7.4	7.4	7.4
Δ°C. . . . .	—0.56	—0.56	—0.56	—0.56	—	—	—	—
Chloride mEq/L . . . . .	82	78	102	102	100	109	440†	335†
Sodium mEq/L . . . . .	83	18.6	140–155	138	140	138	525†	290†
Potassium mEq/L . . . . .	48	95	3.6	4.2	4.8	4.1	9.8†	18.3†
Protein, total g/100 ml. . . . .	20–24	36.8	6.5–7.4	5.9–7.2	1.8	2.1	28†	2.8–3.6
Glucose . . . . .	90	74	—	97	—	—	70	136
Lipids . . . . .	560	600	530	—	—	—	—	200–7,000
Water . . . . .	83	72	94	93	98	95–99	—	—

\*mg/100 ml. r.b.c.

†mg/100 ml.

potentially high nutritional level. In mammalian blood, apart from protein and the usual inorganic constituents, the substances likely to be of physiological importance to a parasite are fat in the form of neutral fats (triglycerides), lecithin and cholesterol, amino acids and carbohydrates. Although the greater part of the amino acids are deaminated by the liver, some pass into the systemic circulation from which they are normally taken up by the tissues to repair protein wear and tear. The resting level of amino acids in blood (man) is 3–5 mg per 100 ml, but may rise to 10 mg per 100 ml after a protein meal. Glucose in the blood of a fasting animal may be 50–100 mg per 100 ml but may rise rapidly after feeding to 125 mg per 100 ml, to fall again as it

diffuses into the tissues for energy purposes. Blood parasites have at their disposal complex protein molecules, both within the blood cells (e.g. haemoglobin) and in the serum. Serum proteins were originally separated into two fractions, albumin and globulin, a division based on their solubility in strong saline solutions. With modern techniques, serum proteins are separated electrophoretically into five components—albumin, two alpha globulins, one beta globulin and a gamma globulin. Globulins, particularly gamma globulin, are concerned in immunity reactions and are considered further elsewhere (Chapter XXXII). Only a limited number of parasites are capable of the direct utilisation of blood protein. The trematode *Schistosoma* has already been referred to above. The malarial organism (*Plasmodium* sp.), which metabolises haemoglobin within an erythrocyte, is a further example.

The gaseous content of the blood varies with the species and the source (i.e. whether arterial or venous; see Table 1). In man, the difference between the oxygen tension in arterial and venous blood shows a utilisation of 27 per cent, whereas in animals with a high metabolic rate (birds) it may be as high as 60 per cent. The carbon-dioxide content is remarkably constant at about 40 mm Hg, and the pH varies only slightly on each side of neutrality with an average of 7·4 in most warm-blooded animals. The relative viscosity is about 1·8.

### 2.3 Reticulo-Endothelial System (R.E.S.)

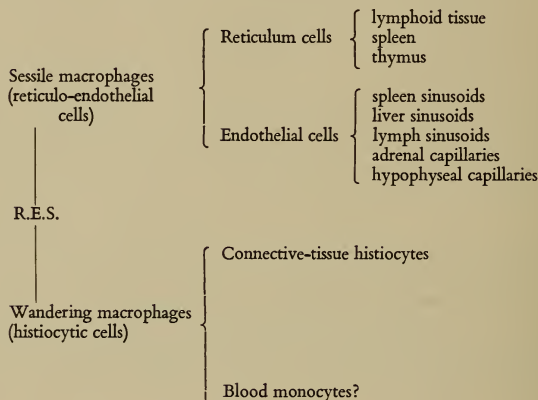
The reticulo-endothelial system is of special interest to the parasitologist, for not only does it serve as a habitat for certain protozoans, but it plays an important part in the defence mechanism of the body by phagocytic action and the production of antibodies. The characteristic cell of the system is the *macrophage* and the system is sometimes referred to as the *lymphoid-macrophage* system. A macrophage is characterised by its relatively large size, ability to migrate throughout the body and to ingest and digest foreign particles.

The cells of the R.E.S. fall into two main classes (Table 7):

(a) *Wandering* macrophages (histiocytes) which occur in connective tissue throughout the body.

(b) *Sessile* macrophages (reticulo-endothelial cells) which are cells of the supporting network tissue (reticular) and the endothelial (channel-lining) tissue of a number of organs especially the liver, spleen, lymphoid tissue and bone marrow. These cells are often spoken of as *fixed tissue cells*, for their locations are fixed, but they occur in strategic positions along the finer blood and lymph channels where they are in a position to

TABLE 7  
OCCURRENCE AND DISTRIBUTION OF CELLS OF THE RETICULO-  
ENDOTHELIAL SYSTEM (R.E.S.)



phagocytose foreign materials carried to them. In mammals, the largest concentration of macrophages occurs in the spleen and in birds in the liver (Küpfér cells).

Little is known concerning the environmental conditions within the cells of the R.E.S. They patently contain powerful digestive enzymes which attack and break down most foreign materials. Certain blood parasites (*Plasmodium* and *Leishmania*) are unaffected by these enzymes and can multiply within the cells of the R.E.S.

#### 2.4 Tissues and Other Habitats

*Muscles.* Muscular tissue has some properties advantageous to the parasitic mode of life and is a site for both protozoans and helminths. It is well supplied with blood so that easy transport to the final site is available. It is rich in nutrients especially carbohydrates, and it contains abundant oxygen (0.7–1 per cent). On the other hand, due to lactic-acid, production, its pH and osmotic pressure are liable to considerable and sudden fluctuation. The same is likewise true of its oxygen consumption which may rise thirty-fold with increased activity; the CO<sub>2</sub> output will also rise proportionally. It is difficult to estimate the availability of contained oxygen as presumably there will be competition between parasite and tissue for available supplies.



*Liver.* Protozoans (e.g. coccidians) and larval helminths (especially trematodes and larval cestodes) are the main parasites found in the liver. This organ does not provide a very stable environment, for its chemical composition may fluctuate widely depending on whether the diet is a balanced one, or one rich in proteins, fats or carbohydrates. Ranges for extremes are: fat, 1·6–52 per cent; glycogen, 0·07–11 per cent; water 35–73 per cent of liver weight. It is also rich in accessory growth factors, such as iron or vitamins and plasma proteins. Its arterial and venous supply is excellent so that as a tissue habitat it is probably aerobic. From the abundance of parasites in or near the liver, it may be assumed that as an environment it is particularly favourable, and that many of the stored materials are available for absorption by the parasites. Its pH is in the region of 7·0 with a tendency towards acidity.

*Body Cavity (Peritoneal Cavity).* Many larval helminths and several acarines (e.g. *Pentastomida*) occur commonly in the peritoneal cavity of mammals, especially rodents. In fish, too, this habitat is a favourable one for certain larval helminths. Little is known of the physical and chemical conditions within the cavity. The oxygen tension in rabbits, rats, guinea pigs, cats and monkeys, is within the range 28–40 mm. Hg and it might be termed a semi-anaerobic environment. The body cavity is kept moist by the peritoneal (ascitic) fluid, essentially a transudate of blood, from which it differs only slightly (Table 6). One of the major differences is the smaller amount of protein, 2·1 g/100 ml against 6·5–7·4 g/100 ml in plasma.

*Cerebro-spinal fluid.* The chemical composition of the cerebro-spinal fluid somewhat resembles that of lymph. It is apparently a true secretion and not just a dialysate of blood plasma. It differs from other body fluids in the small protein content, 28 mg/100 ml or approximately 0·3–0·5 per cent that of blood plasma. There is a correspondingly small amount of antibody content, with the result that parasites may be able to persist in the C.N.S. after antibodies have eliminated them from other parts of the body.

## 2.5 Invertebrate Habitats

Although invertebrates are parasitised by adult forms, their main interest to the parasitologist lies in the fact that they serve as intermediate hosts to all the major parasitic groups—Protozoa, Trematoda, Cestoda, Nematoda and Acanthocephala.

The most favoured sites are probably the haemocoel, the coelom, the digestive gland or the muscles. To discuss the nature and composition of all these habitats in the arthropods and molluscs—the groups which act chiefly as intermediate hosts—is beyond the scope of this book. With the exception of the insects, the properties of the coelom, digestive gland and other possible sites are very poorly known for the majority of

groups. The nature and properties of the insect gut and the composition of insect blood have been widely studied and some excellent reviews are available (Buck, 1953; Day and Waterhouse, 1953a, b, c; Waterhouse and Day, 1953). It is worth while noting that in the insect blood 50–85 per cent of the non-protein nitrogen is in the form of free amino acids—a concentration nearly fifty times that in human serum. In some pupae it may reach 20 g/l. The arthropod haemocoel in general is thus likely to present an environment particularly suitable for the growth of organisms such as encysted helminth larvae, which are enzymatically incapable of dealing with complex protein materials, and entirely dependent on nutriment diffusing through the cyst membranes. It is not surprising to find that such larvae occasionally exhibit neoteny.

## 2.6 Importance of Nutritional Levels of Environment in the Life Cycles of Parasites

It is well recognised that the nutritional demands of an organism may vary with different phases in its life cycle. Thus, the demands of a cysticercus stage of a cestode, undergoing growth in size with little differentiation, are likely to be different from those of an adult cestode producing thousands of eggs daily. Two extreme levels of nutrition can be recognised, a 'survival' level at which cells or tissues metabolise without undergoing growth in size or differentiation, and a 'differentiation' level at which cells can undergo growth and differentiation into more complex structures such as eggs or sperm. At a survival level, the available nutrition is adequate merely to satisfy the energy demands of metabolism and to provide the materials for protein turnover and replacement. At a differentiation level, the nutrition is additionally able to satisfy the higher energy requirements and material demands of complex synthetic processes. In the case of the egg-producing cestode referred to above, these synthetic demands are often of a very high order.

In natural life cycles, then, it is probable that nutritional barriers can similarly operate. This implies that the degree of differentiation, the rate of development, or the reproductive capacity of a parasite will be dependent, amongst other factors, on the nutritional value of the particular environment, especially as regards the availability of protein. In general, the metabolic demands of parasites of homoiotherms will be higher than those of poikilotherms. Marked differences exist, however, between species. For example, the egg production of the trematode *Schistosoma japonica*, at 500 eggs daily, is one hundred times that of the related species *S. mansoni*, although both inhabit the portal system of mammals. Some parasites of homoiothermic vertebrates,

such as filariae, have become adapted to environments with a low nutritional level, e.g. the coelom, and in general, the maturation time of these forms extends over many months.

*In vitro* studies, discussed further in Chapter XXXV (p. 409), bear out this view. In media of 'low' nutritional value, the larvae of certain trematodes and cestodes may 'survive' without differentiation for prolonged periods, whereas if the nutritional value of the medium is raised, growth and differentiation of the larvae can take place. There is some evidence from recent experimental work on cestodes and trematodes to suggest that under favourable cultural conditions, in highly nutritional media, maturation *in vitro* can be even more rapid than *in vivo*.

In considering helminth life cycles, at least, a knowledge of the properties of the environments of the intermediate host could enable the possible level of organogeny, to which a larval stage of a particular species could develop, to be predicted. For example, if an adult trematode has a metabolic demand of  $x$  units of protein per day when producing eggs, it cannot do so if the environment only has available  $x - y$  units per day. Hence, if the protein content falls below this level, the egg-producing level of organisation cannot be reached. This hypothesis therefore implies that there are nutritional barriers to maturation. The same principle will clearly apply in the case of other metabolic materials such as carbohydrates, vitamins, growth factors, etc.

This is a somewhat over-simplified view of the factors underlying developmental patterns in helminth life cycles, but it perhaps enables such cycles to be considered in a new light. It does not imply that nutritional factors are the only external factors regulating differentiation and maturation of parasites in their hosts. In many—perhaps most—cases, special stimuli are required by the parasite before it is metabolically or morphologically capable of maturation, and unless these stimuli have been applied, the nutritional factors are unable to operate. Thus, maturation of several protozoans (p. 115) and one trematode (p. 130) has been shown to be dependent on the endocrine behaviour of the host, and some nematode larvae only exsheath (i.e. become potentially capable of reaching maturity) in response to physico-chemical factors in a particular site in their host (p. 12).

The importance of the nutritional level of the environments in which parasites live is emphasised repeatedly throughout this text, but the general principle can only be accepted as a working hypothesis in this stage of our knowledge. The question is likely to prove to be a complex one dependent on many inter-related factors, and much more experimental work will be required before the processes underlying development in different environments can be determined.

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## CHAPTER III

# THE AMOEBAE

Amoebae occur in almost every natural habitat capable of supporting life—water, soil, decaying plant material and sewage. Organisms with such a wide distribution can be readily ingested accidentally by potential hosts, in drinking water or on plant or animal food material. It is not difficult to visualise how, by suitable adaptations, physiological as well as morphological, some species become at first temporary, and finally permanent parasites of the mouth and intestinal canal. Nearly all phyla of the animal kingdom, both vertebrate and invertebrate, have been found to harbour at least one, and sometimes several species of parasitic amoebae; one, *Entamoeba paulista*, even occurs as a hyperparasite in a parasitic ciliate, *Zelleriella* sp.

Amoebae are essentially scavengers; they live mainly on bacteria or food detritus of bacterial dimensions, with an occasional 'rogue' showing tendencies towards carnivorous habits and attacking the tissue of its host. Their pseudopodial feeding habits impose a peculiar limitation on their habitat, namely, to be suitable, it must present a surface of some kind on which the amoebae can move, capture and ingest prey. This limitation is probably one of the most important controlling the distribution of amoebae in the body. A habitat such as the blood stream, in which the environmental current (i.e. the blood stream) is strong, would be unlikely to present suitable conditions for a potentially parasitic amoeba; bacteria, which form the diet for most species of parasitic amoebae, would also be absent. The most virulent species of human amoebae, *Entamoeba histolytica*, can, however, invade the portal blood stream and may find suitable conditions for growth and multiplication in the liver. It is now known that bacteria, or some metabolite they produce, are *essential* for normal growth and reproduction of parasitic amoebae. This again imposes a further limitation on their distribution.

In the body of the host, amoebae confine themselves to niches which present suitable surfaces on which they may browse and which are rich in bacteria and decaying food material. In vertebrates, these conditions are provided by the mouth, the large



intestine, and the caecum, and it is in these sites that the majority of species occur. It is significant that the upper region of the small intestine, which is virtually sterile, is free from amoebae. In invertebrates they are likewise confined to the alimentary canal. Physical and chemical conditions of the intestinal environment, pH, O<sub>2</sub> tension, pCO<sub>2</sub>, osmotic pressure, oxidation-reduction potential, etc., must also play some part in limiting their distribution.

### 3.1 Type Example: *Entamoeba muris*

Definitive hosts: mouse, rat.

Location: caecum and large intestine.

Transmission: cysts.

*General Morphology of Trophozoite.* The most complete account available is that of Neal (1950). The general form of the active trophozoite is irregular (Fig. 2 c) and about 12–30  $\mu$  in length. There may be two 'races', a 'small' race in mice, and a 'large' race in rats. The ectoplasm is usually readily distinguished from the endoplasm. The pseudopodia are ectoplasmic, that is, they are extensions of the ectoplasm without corresponding endoplasmic streaming, such as occurs in most free-living amoebae. In fixed preparations, the ectoplasm is homogeneous and finely granular, and the endoplasm coarsely granular with food vacuoles and nucleus. The nucleus is characterised by a peripheral layer of chromatin in the form of a beaded ring (Fig. 2 c) and an eccentric karyosome. The peripheral concentration of chromatin is characteristic of parasitic species of *Entamoeba*, and this feature may be used to distinguish it from other genera. The following stages may be recognised:

- (a) trophozoites—the mature adults, large and active.
- (b) precystic stages—small amoeboid stages prior to encystment.
- (c) cysts—encysted stages.
- (d) metacystic stages—excysting stages with many nuclei giving rise to (a).

Except for *Entamoeba gingivalis*, which does not form cysts, departures from this pattern of development are unusual amongst parasitic amoebae.

*Reproduction.* This is by binary fission, the nucleus dividing some time before the cytoplasm. The dividing nuclei are characterised by the appearance of chromatin granules with the loss of peripheral beading; whether these granules represent 'chromosomes' is open to question.

*Encystation.* In *E. muris*, the conditions under which encystation takes place are not fully understood. The precystic forms differ from the trophic forms in (a) their smaller

size and (b) the fact that inclusions are not found, which results in the demarcation between ectoplasm and endoplasm being less clear. The organisms become rounded and secrete a cyst wall, probably composed of a keratin or an elastin-like albuminoid; three successive nuclear divisions give a cyst with eight nuclei. These are only faintly visible in the living cyst with the light microscope, but are more clearly seen after staining with iodine. A characteristic of the newly formed cyst is the appearance of a *glycogen*

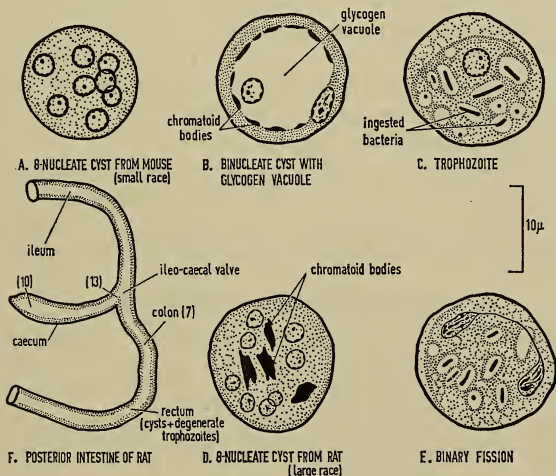


FIG. 2. A-E. Morphology of *Entamoeba muris*. F. Its distribution in thirteen autopsies; the numbers in brackets refer to the number of rats with infections at each site (after Neal, 1950).

*vacuole* (Fig. 2 B), a large vacuole which reaches its maximum size at the binucleate stage, and which almost fills the cyst.

These vacuoles are characteristic of most parasitic amoebae in the cystic stage, and presumably serve as food reserves. During division, the vacuole gradually diminishes so that by the time the eight-nucleate-cyst stage is reached it has virtually disappeared. The glycogen vacuole is readily revealed in iodine-stained cysts, or in fixed preparations, by any of the standard histochemical methods for glycogen (P.A.S. or Best's carmine).

A cyst usually contains, in addition, refractive rod-like structures called *chromotoid bodies* or *chromidial bars*, generally in the form of bars with pointed ends. These may be absent from the mature cysts but when present may be of any shape, but are usually bar-shaped with irregular, splintered or less frequently, rounded ends.

The cyst size varies somewhat, and there is evidence in this species for the existence of several races, all capable of infecting rats and mice and probably hamsters as well; typical measurements are: small race from mice, 9–19  $\mu$  (Fig. 2 A); large race from rats, 12–22  $\mu$  (Fig. 2 D).

*Life cycle.* Cysts are ingested with food or drinking water, or reach the mouth accidentally, and hatch in the duodenum. Conditions under which excystation takes place have not been studied in this species, but evidence from other species suggests that anaerobic conditions, the presence of bacteria, certain inorganic ions, glucose and B vitamins may all be involved. After excystation in the intestine, the amoebae ultimately become established in the caecum for which they have a marked predilection; rarely do they occur in quantity higher up the alimentary canal than the ileo-caecal valve, and only in reduced numbers in the colon (Fig. 2 F).

*Nutrition.* Like the majority of amoebae, *E. muris* is a non-pathogenic species. It is essentially a scavenger, feeding on whatever materials are at hand, and its food vacuoles normally contain bacteria or intestinal protozoans (commonly trichomonads), but any other objects, such as blood cells, if such are free in the gut lumen, may be ingested.

### 3.2 Entamoebae of Man and Related Species

Three species of the genus *Entamoeba* infect man, *E. histolytica*, a potentially pathogenic form in the caecum and colon; *E. coli*, in the same sites; and *E. gingivalis* which occurs in the mouth. These species, as well as those from many other animals, may readily be cultured through all their stages of development *in vitro*. Morphological and physiological characteristics of some of these species are given in Table 8.

#### 3.21 *Entamoeba histolytica*

The term 'histolytica' literally means 'tissue-dissolving'—a term referring to its carnivorous habits. It is an amoeba with a world-wide distribution; a high incidence is reached in some populations. The host-parasite relationship of this organism presents one of the most remarkable enigmas in parasitology, for, although it is known to be a pathogen, some 80 per cent of persons infected show no trace of disease.

*Morphology.* Due to the fact that two races of this species are believed to exist, descriptions of the morphology have been somewhat confusing. The most recent analysis of the question (Burrows, 1957) concluded that there exists:

- (a) a 'large' race, *Entamoeba histolytica* (or *Entamoeba histolytica histolytica* of some workers) with trophozoites in the size range 7–40  $\mu$ . Trophozoites fall into two groups—'minuta' trophozoites with a size range of 7·0–15·9  $\mu$  and 'magna'



TABLE 8

## COMPARISON OF SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SPECIES OF ENTAMOEBÆ

	<i>E. muris</i>	<i>E. coli</i>	<i>E. histolytica</i>	<i>E. gingivalis</i>	<i>E. invadens</i>	<i>E. moshkovski</i>
Host	Mouse	Man	Man	Man	Reptiles	Sewage
Trophozoite size	12-30 $\mu$	20-30 $\mu$	7.0-15.9 $\mu$ ('minuta')* 20-40 $\mu$ ('magna')* 5-11 $\mu$ ('hartmanni')*	10-20 $\mu$	9-38 $\mu$	9-29 $\mu$
Karyosome position	eccentric	eccentric	central; varies	central	eccentric	central
Pseudopodium	sluggish, blunt	sluggish, blunt	blade-like, explosive	broad, active	blade-like, explosive	limax type
Cyst size	9-22 $\mu$	10-30 $\mu$	6.4-15.2 $\mu$ ('minuta')* 3.8-9.3 $\mu$ ('hartmanni')*	cysts not formed	11-20 $\mu$	7-16 $\mu$
Chromatoid bodies in cysts	bar-like, irregular, or splinter-like	sometimes present, splinter-like	often present, bar-like	—	often present, bar-like	often present, bar-like
Nuclei in cysts	usually 8	usually 8	usually 4	—	usually 4	usually 4
Optimum temperature for development	37° C.	37° C.	37° C.	37° C. (?)	24-30° C.	24° C.
Survival temperature range	?	?	32-41° C.	?	16-35° C.	17-37° C.

\* See text.

trophozoites with a size range of 20–40  $\mu$ . Only the 'minuta' trophozoites form cysts (6.4–15.2  $\mu$ ).

- (b) a 'small' race, *Entamoeba hartmanni* (or *Entamoeba histolytica hartmanni* of some workers), with trophozoites 5–11  $\mu$  and forming cysts (3.8–9.3  $\mu$ ).

According to Burrows (1957), *E. hartmanni* can be distinguished morphologically from

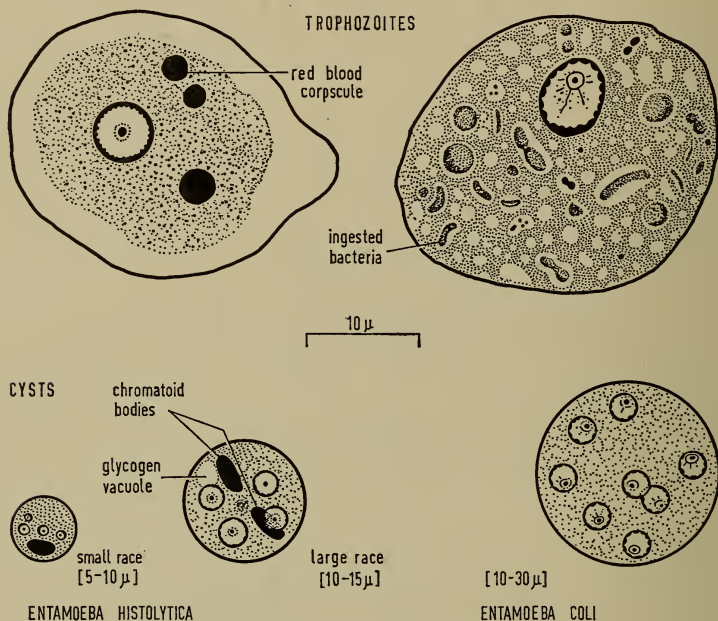


FIG. 3. Comparison of trophozoites and cysts of *Entamoeba histolytica* and *E. coli*.  
(from Hoare, 1949; after Dobell and O'Connor, 1921).

*E. histolytica* by the size and structure of the trophozoite, by the size of the cyst nuclei and by the ratio of nuclear diameter to cyst diameter in uninucleate cysts. It remains to be seen whether this view will be substantiated by other workers.

It is believed that the large (*E. histolytica*) and small (*E. hartmanni*) are true races (i.e. hereditarily fixed) which cannot change into the other species of *Entamoeba* (e.g.

*E. invadens*, see p. 35). The general morphology is close to that of *E. muris*, except for a few points. Thus in *E. histolytica* (Fig. 3) the karyosome is usually central; in *E. muris* the chromatin is coarser and the karyosome is eccentric. In the living trophozoite, the nucleus is almost invisible by the light microscope but can be seen by phase-contrast microscopy, whereas in *E. muris*, the nucleus is clearly visible by the light microscope.

The movement of the pseudopodia in *E. histolytica* as seen in a fresh film on a warm stage is striking, the single ectoplasmic pseudopodium moving out in a manner usually described as 'explosive'. The trophozoites are considerably larger than the cysts, and the precystic forms may lay down in their cytoplasm one or two chromatoid bodies which are bar-like as opposed to the irregular or splinter-like form in *E. muris*. In encysted forms, the nucleus divides twice to give four nuclei (Fig. 3) with the same characteristics as those of the adult trophozoites. Glycogen vacuoles are formed in the young cysts.

*Life cycle* (Fig. 4). After ingestion by man, and excystation in the small intestine, the tetranucleate amoebae escape and each produces eight uninucleate amoebae by a process of division. The amoebae may either invade the mucous membrane of the colon and caecum and multiply, or may remain in the intestinal lumen.

*E. histolytica* has long been regarded as an obligatory tissue parasite and the mere presence of cysts in the faeces called for immediate remedial action. Whereas there is no doubt whatsoever that it may be a pathogenic parasite, capable of invading host tissue with the production of lesions and clinical symptoms, the accumulation of evidence (Hoare, 1952, 1958) now suggests that in the majority of persons it is harmless, the host serving as a 'symptomless carrier'. Under certain conditions, not understood at present, amoebae are virulent and invade the intestinal wall with the aid of proteolytic enzymes, penetrating into Lieberkühn's glands, and multiplying there, often penetrating deeper through into the muscularis mucosae. Invading forms may even enter the portal blood stream and be carried to the liver, setting up abscesses there (Fig. 4).

No complete agreement has been reached on the relationship between the harmless and pathogenic forms. The status of *E. hartmanni* is generally accepted as a small non-pathogenic species. The main controversy arises with regard to the large race. It is believed that there exist two strains of this large race, a virulent and an avirulent strain. The virulent strain is distributed mainly in the tropics, whereas the avirulent strain is confined mainly to the temperate zones. The position is complicated by conflicting experimental evidence from different hosts. Thus Beaver *et al.* (1956) have shown that when forty-two human volunteers were infected with cysts from a symptomless carrier, not a single clinical case of amoebiasis resulted. Yet cysts from the same source when

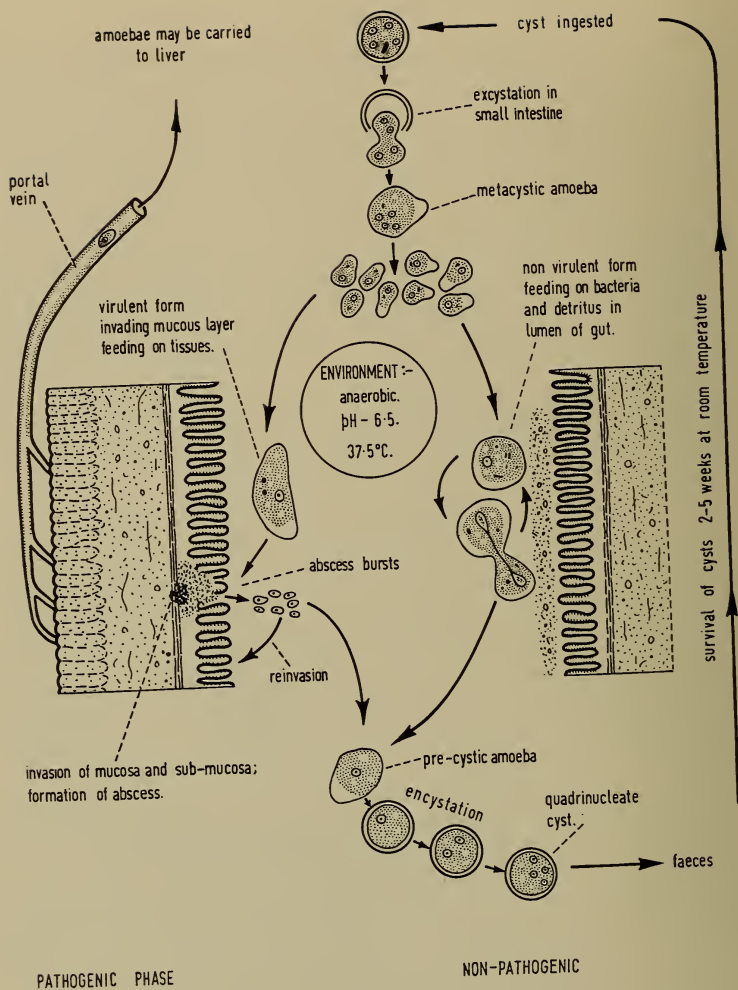


FIG. 4. Life cycle of *Entamoeba histolytica* in man (original).

fed to dogs, guinea pigs and rats produced typical amoebic lesions and were as pathogenic in guinea pigs and rats as a recently isolated strain from a clearly dysenteric patient.

Thus, the so-called virulent strain can become virulent in other hosts and it is evident that factors of an unknown nature are operating in determining the intestinal behaviour of the amoebae. A similar pattern is well known to occur in other protozoans, such as trypanosomes which may be non-pathogenic in their 'natural' hosts but highly pathogenic in 'experimental' hosts (pp. 53, 57).

*E. histolytica* has been cultured *in vitro* under axenic conditions (Shaffer and Frye, 1948; Reeves, Meleney and Frye, 1957), but it is normally dependent on bacteria for the provision of some essential growth factor (see pp. 37, 398).

Recent studies (Phillips *et al.*, 1955) on infections of *E. histolytica* in germ-free guinea pigs have provided striking confirmation of this. In germ-free guinea pigs, the longest period of survival of *E. histolytica* was five days. In guinea pigs which harboured *Escherichia coli* or *Aerobacter aerogenes* as monocontaminants, active ulcerative amoebiasis was produced in all cases. Thus, although *E. histolytica* is unquestionably the causative organism of amoebiasis, responsibility for the disease must be shared with other micro-organisms which contribute to the etiology, pathogenesis and pathology.

### 3.22 Other Species of Entamoebae

*Entamoeba coli*. This is a non-pathogenic species whose distribution is world-wide, occurring in some 30 per cent of the world's population. It is found in the large intestine and distinguished from *E. histolytica* by a number of features, chief of which are: (a) its slower movement; (b) its pseudopodium is mainly coarser and not clear like *histolytica*; (c) its nucleus is coarser and with an eccentric karyosome (Fig. 3); (d) it has a larger number of food vacuoles.

The precystic stages are difficult to distinguish from those of *E. histolytica*, critical observation of the nucleus being necessary. The cysts have eight nuclei and the chromatoid bodies, when present, are splinter-like but never bar-like (Fig. 3).

*E. coli* is entirely a scavenger, feeding on bacteria and food detritus in the large intestine. It is not capable of eroding the intestinal mucosa, but as an indiscriminate feeder it will phagocytose blood cells, if these are available.

*Entamoeba gingivalis*. A common parasite of the human mouth and the first entozoic amoeba described. The spaces between the teeth and the soft pits of the gums offer ideal surfaces for amoebae, as in these sites bacteria and food detritus abound. *E. gingivalis* resembles *E. histolytica*, has a crystal-clear ectoplasm and moves actively (Fig. 5). Its food vacuoles are usually numerous and contain bacteria, leucocytes and occasionally red cells. The pathogenicity of this species has never been established. It often abounds in cases of pyorrhea, but it may merely find the environmental conditions in these cases



particularly suitable for its own nutritional requirements and may not necessarily be concerned in the production of the pathological condition.

*Entamoeba moshkovskii*. This species is not an animal parasite, but it may be conveniently discussed with the other forms. It is a free-living species, which was first discovered in a sewage-disposal plant in Moscow (Tshalaia, 1941) and later in Brazil and Great Britain

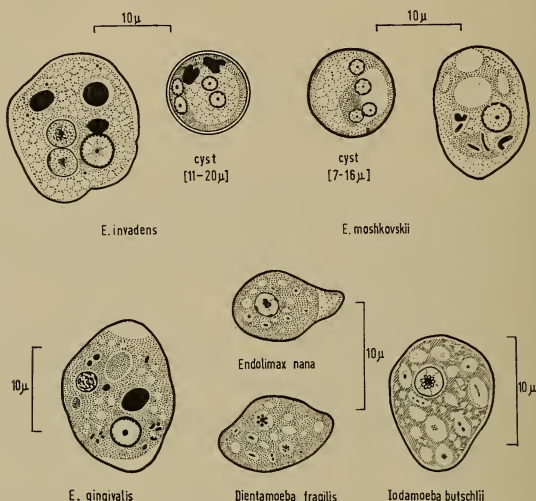


FIG. 5. Trophozoites and cysts of various parasitic entamoebae.

*E. gingivalis*—a non-pathogen from the human mouth;

*E. invadens*—a pathogen from reptiles;

*E. moshkovskii*—a free-living species from sewage.

The remainder are intestinal parasites of man (adapted from various authors).

and is doubtless of world-wide occurrence. Morphologically (Fig. 5) it closely resembles *E. histolytica* throughout its life cycle, including encystment and metacystic development, differing from it only in details (Neal, 1953). Yet all experiments to establish this species in rats or amphibians, animals which might act as host reservoirs in sewage beds, have failed, and there seems no doubt that it is a true free-living form. This is emphasised by its optimum temperature of cultivation, which is 24° C. At 37° C., the obligate temperature for *E. histolytica*, *E. coli* and *E. muris*, the organism grows well

but does not encyst. The temperature requirements in some species are compared in Fig. 6 (McConnachie, 1955).

The osmotic relations of *E. moshkovskii* are of especial interest. In sewage, due to the presence of dissolved materials, the osmotic pressure is liable to be considerably above that of fresh water, though unlikely to be up to the mammalian range of  $\Delta = -0.5$  to  $-0.6^\circ\text{C}$ . Specimens of *E. moshkovskii* from sewage, when placed in tap water, have been reported to produce a number of vacuoles which fill slowly, run together and discharge. It is tempting to call these vacuoles 'contractile vacuoles' as they are clearly attempting to eliminate excess water. Their efficiency is not so high as those of entirely fresh-water amoebae with permanent contractile vacuoles, for the organism gradually dies. The formation of vacuoles is reversible, for tap-water forms containing vacuoles, when cultured *in vitro* in media isotonic with mammalian blood, are reported to develop into forms lacking contractile vacuoles.

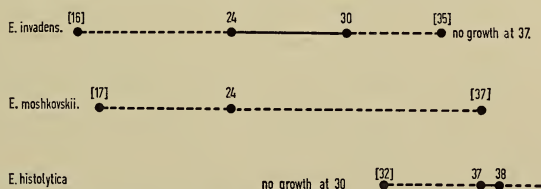


FIG. 6. Temperature requirements of *E. invadens*, *E. moshkovskii* and *E. histolytica*. Figures in  $^\circ\text{C}$ . Figures in parentheses represent maximum or minimum temperatures tested for growth. Figures not in parentheses represent optimum temperatures (after McConnachie, 1955).

*Entamoeba invadens*. *E. invadens* has sprung into prominence within recent years, mainly on account of its morphological resemblance to *E. histolytica*, and the fact that it is the only other known pathogenic amoeba (Geiman and Ratcliff, 1936). It causes spontaneous amoebiasis in lizards and snakes but may be harmless in other reptiles (possibly turtles). The general details of its life history are similar to *E. histolytica*. This species may prove to be a variety of *E. histolytica* adapted for growing at a lower temperature ( $24-30^\circ\text{C}$ . against  $37^\circ\text{C}$ . for *E. histolytica*). It has been cultured under axenic as well as monoxenic conditions (p. 400).

### 3.3 Other Intestinal Amoebae

*Endolimax nana*. A small intestinal amoeba of man, measuring some  $6-15\ \mu$ . It has a wide distribution, occurring in 15-30 per cent of the world's population. The nucleus is characterised by the absence of chromatin granules (Fig. 5) and the presence of a large karyosome, usually irregular in shape and sometimes divided into several parts. The trophozoite is sluggish in movement like *Entamoeba coli* and is similarly a harmless parasite. *Endolimax reynoldsi* from the lizard and *E. blattae* from the cockroach are closely related forms.

*Iodamoeba butschlii*. A small amoeba, very common in monkeys and pigs, up to 50 per cent of the latter being infected in some areas. It also occurs in man, where its incidence in most countries is usually less than 10 per cent. The trophozoites are larger than *E. nana* but smaller than *E. histolytica*. The nucleus is not visible in the living form, but is a striking feature in stained preparations (Fig. 5). The karyosome is large and centrally placed and surrounded by a peripheral layer of lightly staining bodies. Cysts usually have one, but sometimes two or three nuclei, and a large, sharply defined, glycogen vacuole.

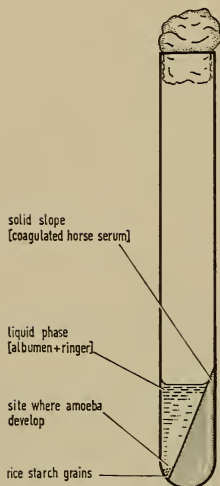


FIG. 7. Tube with Boeck-Drbohlav diphasic medium for *in vitro* culture of intestinal entamoebae, and certain other intestinal protozoans (after Dobell, 1942).

*Dientamoeba fragilis*. Only one species is known, which occurs in monkeys and man (Fig. 5). Although not a common form, it has a world-wide distribution. About 50 per cent of all specimens encountered have two nuclei. The pseudopodia are leaf-like and the endoplasm and ectoplasm sharply defined. The nucleus is not visible in either living or iodine-stained preparations, and can only be seen properly in fixed preparations stained in Heidenhain's haematoxylin.

### 3.4 Physiology of Intestinal Amoebae

Almost all the common species of parasitic amoebae have been cultured more or less successfully in the presence of bacteria. Boeck and Drbohlav (1925) introduced a method using a diphasic medium with added starch (Fig. 7) which has been extensively used. A diphasic medium is not essential, however, and monophasic liquid media such as serum-Ringer, egg-yolk infusion, etc., are wisely used. The problem of *in vitro* culture is discussed further in Chapter XXXIV.

As *in vivo* (p. 33), the presence of micro-organisms is essential for normal growth of parasitic amoebae in culture and in their absence the amoebae die. Bacteria are usually used, but *Trypanosoma cruzi* is also effective although large numbers,  $10^7$ – $10^8$  per millilitre, are required for successful culture. The role played by bacteria is further discussed on p. 399. A number of species, *E. histolytica*, *E. invadens*, *E. ranarum*, have been grown monoxenically.

The problem of investigating the physiology of parasitic amoebae thus has been



seriously hampered by inability, until recently, to establish axenic culture techniques. One approach has been to employ a method of difference to subtract the contribution of the microflora from by-products and processes examined. Conclusions from the use of this approach can only be accepted with the utmost caution as it is frequently not possible to distinguish clearly between contributions from the medium and those of the bacteria, and furthermore, the growth of the bacteria may be influenced by metabolic products from the amoebae.

Within recent years, axenic culture has been achieved in a cell-free medium based on chick-embryo juice; this contains a particular factor which can replace that supplied by the bacteria (Reeves, Meleney and Frye, 1957).

*Physico-chemical considerations.* Few controlled experiments have been carried out on the effect of environmental factors on parasitic amoebae.

*Temperature.* The effect of temperature has been briefly referred to on p. 35, upper and lower temperature limits being known for several species.

*pH.* *E. histolytica* can tolerate wide variations of pH in different culture media, the pH being a function of the interaction between the bacterial flora and substrate. The overall range for which growth has been reported is of the order of 5.4–8.3, although growth is poorer at each end of this range.

*Osmotic pressure.* *E. histolytica* remains viable over a wide range of osmotic pressures ( $\Delta = -0.95$  to  $-0.17^\circ \text{C.}$ ) and similar results have been reported for other intestinal amoebae. There seems no doubt that parasitic amoebae can adjust considerably to fluctuating environmental conditions.

*Biological oxygen relationship.* Both *in vivo* and *in vitro* intestinal amoebae grow in sites which are virtually anaerobic. In the routine cotton-wool-stoppered tubes, growth only takes place at the bottom of the tube (Fig. 7) where anaerobic conditions are most complete. On the other hand, in anaerobically sealed culture, amoebae will grow higher up the walls. *E. histolytica*, at least, and probably the majority of intestinal forms, show a degree of obligatory anaerobiosis approaching that of the most sensitive anaerobes. The reducing activity of a culture medium is clearly related to the oxygen tension. Thus anaerobic organisms proliferate only at low O/R (oxidation-reduction) potentials and it is suggestive that cultures containing bacteria which develop low potentials yield the best amoebic growth, although this result has been disputed. The addition of reducing agents such as cysteine apparently favours the growth of *E. histolytica*—a result possibly due to lowered Eh, although it may act as a nutrient as well.

The Eh of the rat caecum is approximately  $-200$  mV, and average growth is obtained at this level. Much heavier growth, however, is produced at Eh of levels

— 300 to — 500 mV (Fig. 8). There seems little doubt that one of the major functions of the bacteria is to maintain the oxidation-reduction potential at a suitably low level. *General Metabolism.* On account of the failure of early investigators to establish axenic cultures, almost no accurate information regarding the metabolic processes of amoebae is known, and reliance must be placed mainly on indirect evidence. Certain aspects of the physiology are reviewed by Balamuth and Thompson (1955) and Lwoff (1951). Direct visual evidence does, however, clearly show that rice-starch grains are ingested and digested. This fact, taken with their dependence on anaerobic conditions, suggests

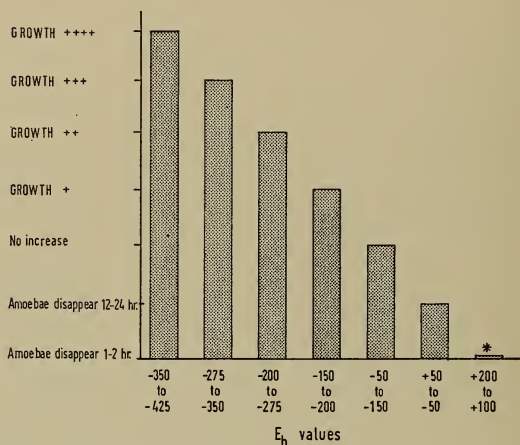


FIG. 8. Relation of oxidation-reduction potentials to growth of *Entamoeba histolytica* after 24-hr. culture (after Chang, 1946). \*1-2-hr. cultures.

a well developed glycolytic mechanism (Table 38) and fermentative activity, but the pathways have not been worked out.

An amylase, a gelatinase and a protease have been detected, but evidence for the occurrence of hyaluronidase in tissue-attacking forms—the presence of which might be predicted on theoretical grounds—is somewhat contradictory. It may be possible that invasive strains may produce this enzyme, but lose the capacity to do so, after being grown *in vitro*.

Nothing is known concerning the protein or fat metabolism of parasitic amoebae.

The role played by the obligate associates, such as bacteria or *Trypanosoma cruzi*, in the nutrition of parasitic amoebae is unknown, although this aspect of their nutrition has been extensively investigated. It would appear that the continuous metabolic activity of the associates produced the necessary growth factor(s) in small amounts, but attempts to isolate this factor(s) have been unsuccessful. There is evidence from penicillin-treated bacteria that ingestion of bacteria fragments is essential for survival (p. 399).

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## CHAPTER IV

### FLAGELLATES:

### INTESTINAL AND RELATED FORMS

Flagellates possess one marked advantage over their amoeboid relatives—they can swim. The possession of flagella enables these protozoans to move actively in environments unsuitable for amoebae. In particular, the dependence on surfaces, so marked in amoebae, is not a feature of the behaviour of the group and in vertebrates they have invaded not only the alimentary canal, but also habitats with a liquid environment such as blood, lymph and cerebro-spinal fluid. In invertebrates, the alimentary canal is the usual habitat.

A further adaptation for life in a liquid habitat is seen in one group, the trypanosomes, in which the body has a streamlined torpedo shape. Locomotion is carried out by one or more flagella; many species also possess an undulating membrane, a protoplasmic membrane united to the body, sometimes bordered by a flagellum. Intestinal species usually have three to five flagella, with the exception of the hyper-flagellates from the intestine of white ants, which have more. Some flagellates are additionally strengthened by the possession of a stiff rod or *axostyle* extending the length of the body.

Flagellates do not, however, only occur in liquid environments, for they have successfully invaded almost every organ of the vertebrate body. Their ability to survive in a number of environments is aided by their ability to change from a flagellated free-swimming to a non-flagellated tissue stage and *vice versa*.

#### 4.1 Classification (after Kudo)

- |          |                 |  |
|----------|-----------------|--|
| Order 1. | Rhizomastigina. | Body amoeboid, with pseudopodia, 1-4 flagella,<br>e.g. <i>Histomonas meleagridis</i> . |
| Order 2. | Protomonadina.  | With 1-2 flagella. No axostyle,<br>e.g. <i>Trypanosoma lewisi</i> .                    |

- Order 3. Polymastigina. Heterogenous group with 3-8 flagella.  
e.g. *Giardia muris*.
- Order 4. Hypermastigina. Numerous flagella; complex organisation. In gut of insects (not considered here).

This is the strictly zoological classification, but it is usual to speak in a general way about 'intestinal flagellates' which occupy the alimentary canal, and 'haemoflagellates' which occur in the blood, lymph or tissues of the vertebrate host. Grouped with the 'intestinal' forms are flagellates which invade other tubular systems, such as the genital tract, and it is convenient to consider them together.

TABLE 9  
COMMON INTESTINAL FLAGELLATES OF MAN AND  
LABORATORY ANIMALS

Species	Host	Habitat
<i>Chilomastix mesnili</i> . . .	man	large intestine
<i>Chilomastix bettencourti</i> . . .	rat, mouse	large intestine
<i>Chilomastix cuculluli</i> . . .	rabbit	large intestine
<i>Chilomastix gallinarum</i> . . .	chicken	caeca
<i>Trichomonas hominis</i> . . .	man	large intestine
<i>Trichomonas tenax</i> . . .	man	mouth
<i>Trichomonas gallinae</i> . . .	pigeon	oesophagus, crop
<i>Trichomonas muris</i> . . .	rat	caecum
<i>Trichomonas foetus</i> . . .	cattle	genital tract
<i>Trichomonas vaginalis</i> . . .	man	genital tract
<i>Trichomonas limacis</i> . . .	slug	intestine
<i>Histomonas meleagridis</i> . . .	turkey, etc.	caeca
<i>Giardia lamblia</i> . . .	man	duodenum
<i>Giardia muris</i> . . .	rat	duodenum
<i>Giardia caviae</i> . . .	guinea pig	duodenum
<i>Giardia duodenalis</i> . . .	rabbit	duodenum
<i>Giardia agilis</i> . . .	tadpole	duodenum

## 4.2 Trichomonads

### 4.21 General Account

**Occurrence.** 'Trichomonad' is a general term used for members of the genus *Trichomonas* which includes a large number of species parasitising a wide range of both vertebrate and invertebrate hosts (see Table 9). Species most readily obtained for laboratory study are *Trichomonas muris*, which occurs in a high proportion of laboratory mice, and *T. gallinae* from the crop of the pigeon. The morphology of species in man and a number of animals has been reviewed by Wenrich (1944).

**Morphology.** Only a general description of a typical trichomonad (based on *T. muris*,

Fig. 9) is given here. Special details of particular species are outlined below. A trichomonad has an ovoid body with a single ovoid nucleus. Its most striking feature and one most easily visible in warm fresh preparations of active forms, is the presence of the free anterior flagella which number three to five, and one flagellum running backwards. The pellicle is extended out on one side to form a frill-like membrane, the undulating membrane, bearing the backward-directed flagellum attached to its outer margin. The movements of this marginal flagellum are translated to the membrane, which thus acts as a supplementary locomotory organ. The undulating membrane has a deeply staining

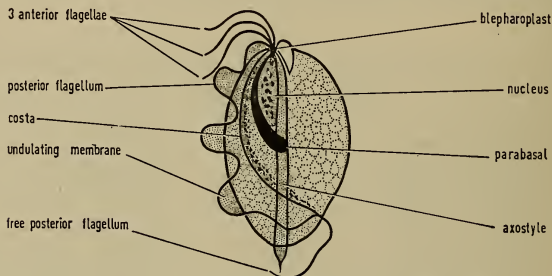


FIG. 9. *Trichomonas muris* from the caecum of the rat. A typical trichomonad (after Hegner *et al.*, 1938).

basal rod, the *costa*, along the line of its attachment to the body. The body is supported by a stiff rod or *axostyle*, which passes through the middle of the body and protrudes posteriorly like a tail. Most species have a sausage-shaped *parabasal* body, situated near the nucleus, with a posteriorly-directed parabasal fibre. There is usually a well-developed cytostome through which solid food, such as bacteria, may be ingested, but it is vestigial in some species.

*Locomotion.* Trichomonads swim with a characteristic wobbly movement, quite unmistakable once observed.

*Reproduction.* By binary longitudinal fission.

*Habitat.* Trichomonads occur in a great variety of environments, a feature possibly related to the possession of several flagella, and also to the fact that, unlike the amoebae, whose nutrition is predominantly holozoic, their nutrition is both holozoic and saprozoic. Although the majority occur in the caecum and large intestine, the mouth, throat, oesophagus, crop, vagina, prostate and uterus are favourable sites for some species.



*Transmission.* There is no evidence that any species is capable of forming cysts, although some species become rounded and lose their flagella; in this condition they appear to be as resistant as a cyst, although a protective wall, characteristic of a cyst, is lacking.

#### 4.22 Particular Species of Trichomonads

*Trichomonas vaginalis* (Fig. 10). This species occurs in the human vagina. It has a world-wide distribution, with an incidence of about 20–40 per cent. It may occasionally be found beneath the prepuce or in the anterior urethra of males. Biologically it is an

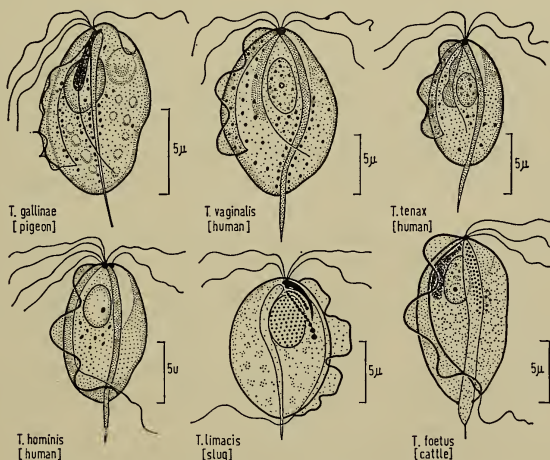


FIG. 10. Trichomonads from man and lower animals (after various authors).

interesting species, as it requires a mildly acid medium ( $pH$  about 5.4–6.0) for development, but since the vagina is normally at a  $pH$  of about 4.0–4.5, the organism does not usually occur. The  $pH$  is maintained by the production of lactic acid by *Lactobacillus* (Doderlein's bacillus) so that only when the balance maintained between this organism and the vaginal secretions is upset, can *Trichomonas* thrive. There is some evidence that its presence produces an inflammatory reaction in the vaginal mucosa, but the pathogenicity has never been satisfactorily demonstrated. Trussell (1947) has summarised our knowledge of this organism.

It is a large form for a trichomonad, up to  $13\ \mu$ , rounded or oval, with five flagella with groups of blepharoplasts. The undulating membrane is shorter than in other trichomonads of man and seldom extends beyond one or two-thirds of the body length. The axostyle is slender, and the parabasal body well developed.

*Trichomonas tenax* (Fig. 10). (Synonym. *T. buccalis*). This species occurs in the human mouth with an incidence of 11–25 per cent. Its presence tends to be associated with caries, pyorrhoea or other infections of the gums, but its role as a pathogen is unproven. It is smaller than *T. vaginalis*, measuring  $5\text{--}12\ \mu$  in length. The undulating membrane is longer than *T. vaginalis* and extends to the posterior border of the body.

*Trichomonas hominis* (Fig. 10). The commonest intestinal flagellate of man. The size range is  $5\text{--}14\ \mu$  but usually about  $8\ \mu$ . It is readily distinguished from *T. tenax* and *T. vaginalis* by the fact that the undulating membrane runs the whole length of the body with the flagellum along its margin becoming free posteriorly. The nucleus is oval. The number of flagella vary; the majority of specimens possess five forwardly directed flagella but some have only three or four. The axostyle is relatively thick, and more curved than in *T. tenax* or *T. vaginalis*. It occurs in the large intestine and is probably non-pathogenic.

*Trichomonas muris* (Fig. 9). A species readily obtainable from the caecum of rats. It shows the characteristics of the genus more distinctly than the human species. There are three anterior flagella, and a posterior flagellum which runs along the undulating membrane and extends posteriorly beyond it. The blepharoplast and axostyle are well-developed.

*Trichomonas gallinae* (Fig. 10). (Often incorrectly referred to as *T. columbae*.) A species common to a number of birds, including turkeys and chickens, but the common or domestic pigeon is probably the natural host. It occurs in the pre-gastric alimentary canal—the mouth, pharyngeal region, oesophagus and crop—and never in the post-gastric regions. From these sites it may invade the tissues of the head and neck and even the lungs, liver and pancreas, but various strains seem to favour different localities. In severe infections it may cause lesions which may prove fatal. The organisms sometimes cause heavy losses in aviaries or poultry farms (Stabler, 1954).

In the pigeon, infection approaches 100 per cent, for the mode of transmission from parent to young is exceptionally efficient. The pigeon's 'milk' in the crop is a favourite habitat for the organism, and a drop is usually teeming with active trophozoites. Within minutes of hatching, a newly-hatched offspring may become infected by transference of milk from the parent bird.

The size is within the range of  $6\text{--}18\ \mu$ ; there are four anterior flagella; the axostyle



is narrow; the undulating membrane is  $\frac{1}{2}-\frac{3}{4}$  the body length and the marginal filament does not continue as a free posterior flagellum.

It may be cultured *in vitro* in a variety of media (Diamond, 1954). The biology has been reviewed in detail by Stabler (1954).

*Trichomonas foetus*. A widely distributed pathogenic species occurring in the genital tract of cattle and of considerable economic importance. It is transmitted venereally from infected bulls to heifers, in which it attacks the vagina and uterus causing abortion and other pathogenic disturbances. The females are self-curing, but the males are apparently infected for life. Research on this organism has been reviewed by Morgan (1947).

*Trichomonas limacis* (Fig. 10). A species occurring in the intestine and liver of the European slug *Limax flavus*. It has the typical trichomonad structure; the posterior flagellum is long and trailing (Kozloff, 1945).

### 4.3 Intestinal Flagellates other than Trichomonads

*Embodomonas intestinalis* (Fig. 11). A rare human parasite with an ovoid body, 5–6  $\mu$ , with two flagella, producing small pear-shaped cysts.

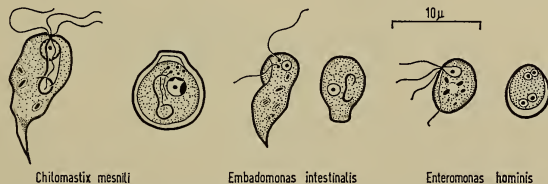


FIG. 11. Trophozoites (left) and cysts (right) of the smaller and rarer flagellates of man (after Hoare, 1949 and various authors).

*Enteromonas hominis* (Fig. 11). Another rare form with an ovoid body, 4–10  $\mu$  in length, with two flagella. Produces elongate oval cysts.

*Chilomastix mesnili* (Fig. 11). The largest intestinal flagellate of man, 6–20  $\mu$  in size. Characterised especially by the large cystostome in the form of a slightly spiral groove whose lateral margins are supported by two filaments. The nucleus is at the extreme anterior end. There are three anterior flagella springing from blepharoplasts and a fourth lies in the oral groove. It produces pear-shaped cysts resembling those of *Embodomonas*, with a characteristically coiled darkly stained filament.

*Chilomastix* occurs in the large intestine; incidence about 1-10 per cent. Numerous species have been reported from other animals.

Genus *Giardia*. A flagellate of this genus is quite unlike any of the preceding species in shape or habits. It has been described in front view as looking like a 'tennis racquet without a handle', and it has a comical, face-like appearance (Fig. 12). It lacks a cytostome, so that nutrition is entirely saprozoic. It possesses two depressions or sucking-discs, by means of which it holds on firmly to the intestinal cells (Fig. 12), clearly an adaptation related to its saprozoic mode of nutrition. It exhibits perfect bilateral symmetry, and there are a double set of nuclei and a complicated arrangement of flagella and blepharoplasts as shown in Fig. 12. In a fresh smear it can be seen to sway from side to side as it swims, its four pairs of flagella lashing.

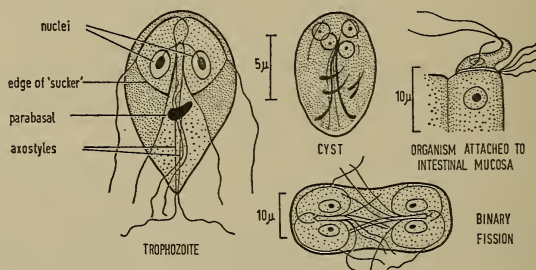


FIG. 12. Various stages of *Giardia lamblia* from the human duodenum (after various authors).

*Encystation*. Unlike trichomonads, species of *Giardia* produce characteristic oval cysts with thick walls (Fig. 12); the remains of the disintegrated flagella forming a central 'streak' visible in iodine or MIF (merthiolate-iodine-formaldehyde) preparations.

*Habitat and nutrition*. Species of *Giardia* are confined in their distribution to the small intestine, particularly the duodenum, occasionally invading the bile ducts. In severe infections, they may carpet large areas of the mucosa. The duodenum is nutritionally the richest habitat in the alimentary canal, containing amino acids, monosaccharides and fatty acids (p. 16) with an environment difficult to reproduce artificially *in vitro*. It is not surprising, therefore, that *Giardia* is one of the few intestinal protozoa which has resisted attempts to culture it. All the intestinal forms readily culturable (*Entamoeba*, *Trichomonas*, etc.) are inhabitants of the large intestine, a low-level nutritional environment with properties not difficult to simulate (p. 397). Since *Giardia* is found closely

attached to the mucosa it is likely that, in addition to absorbing soluble materials from the duodenal contents, it is capable of obtaining nutriment directly from the mucosal cells.

*G. muris* (Fig. 13). The trophozoites of this species occur in a considerable proportion of laboratory rats and mice. Mobile and encysting forms are found in the small intestine; cysts occur in the caecum and faeces.

*G. lamblia* (Fig. 12). This is the species infecting man; it has an incidence of 1-16 per cent. Children seem especially susceptible and mass infections occasionally break out in nursery schools. The pathogenicity has been long disputed, but the general consensus of opinion is that it is a pathogen, and the terms 'lambliasis', 'giardiasis', or 'flagellate diarrhoea' are used to describe the condition brought about by its presence.

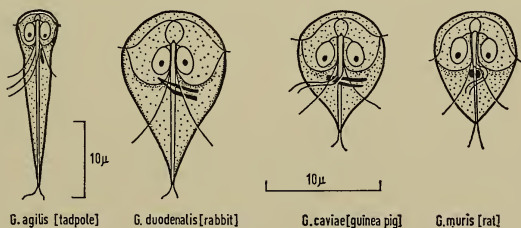


FIG. 13. Various species of *Giardia* from lower animals (after Hegner *et al.*, 1938).

There seems little doubt that the presence of large numbers of *Giardia* in the duodenum could effectively absorb a proportion of the available food materials released by digestion. Also, since large areas of the mucosa might be covered by the organisms, the mucosal cells would have difficulty in carrying out their normal absorptive functions. The effects caused by the parasite may thus have mainly a nutritional basis.

*Histomonas meleagridis*. A small flagellate parasite of the turkey, fowl, quail and ruffed grouse. Its size range is between 8-20  $\mu$ . Its appearance shows considerable variation. In general, it has a rounded form showing sluggish activity on warming. There is usually only a single flagellum but large individuals with two to four flagella sometimes occur. There is a single nucleus, and blepharoplast.

*Histomonas meleagridis* is parasitic in the caeca from which site it migrates through the wall forcing itself even beyond the muscular layers causing characteristic lesions associated with the disease histomoniasis, often known as 'blackhead' by turkey keepers.

As in the case of *Entamoeba histolytica*, it may be carried via the portal vein to the liver where it causes lesions. Although *H. meleagridis* invades the caecal tissues it is essentially an extracellular, not an intracellular parasite.

The transmission of the flagellate is not understood. It does not form cysts and cannot survive longer than several hours in the faeces. Under natural conditions oral ingestion of infected droppings may cause infection but ingestion of infected material from the liver or caeca frequently fails to establish an infection in the laboratory. The organism can be transmitted by subcutaneous or rectal inoculation.

Under natural conditions, *H. meleagridis* is transmitted by oral ingestion of the eggs of the ascaroid nematode *Heterakis gallinae*, from infected turkeys or fowl. The incidence of infected eggs of *H. gallinae*, at least in Britain, is so high that it is difficult to obtain eggs which will not induce histomoniasis in turkeys. The eggs retain their ability to transmit *H. meleagridis* for at least six months and probably longer. The means whereby the nematode eggs transmit this flagellate are quite unknown, for *no stage of Histomonas meleagridis has ever been observed in the eggs or within the larvae developed from the egg!* This presents one of the outstanding mysteries of parasitology. Some workers believe that *H. meleagridis* is never itself present in the eggs of the nematode, but that a *virus* closely associated with *Histomonas* occurs there, and that this virus is the true cause of histomoniasis. On this view, the worms and the flagellate are virtually inseparable in the host, and the apparent pathological effects of the protozoon may be largely due to the virus. This fascinating hypothesis has yet to receive support from experimental data. *Histomonas* may be readily cultured *in vitro* (p. 400).

#### 4.4 Physiology of Intestinal Flagellates

*In vitro cultivation.* *In vivo*, the nutrition is predominantly holozoic and intestinal forms feed on detritus, bacteria and yeast; buccal and vaginal forms feed probably on leucocytes. All species can feed saprozoically and most of the common species have been cultured *in vitro* under axenic conditions. Diphasic media, similar to that employed for parasitic amoebae (p. 400) give satisfactory results. The introduction of antibiotics has greatly assisted the isolation of axenic strains and a number of suitable methods have been developed (de Carneri, 1956). The subject of trichomonad nutrition has been reviewed by Lwoff (1951) and Read (1957).

*Physico-chemical considerations.* Most trichomonads have a multiplication optimum of 37° C., but there are considerable strain differences. The limiting range is about 32–40° C. The minimal time required for one division is 5–7 hrs. (at 35–39° C.).

Trichomonads vary in their sensitivity to pH. In unbuffered media, the pH drops

rapidly due to acid production and death usually results. The optimum pH range for *T. foetus* is 7.0–7.6; *T. vaginalis*, 5.4–6.0 and *T. gallinae*, 6.5–7.5.

As in the case of parasitic amoebae, the oxidation-reduction potential is an important controlling factor in metabolism. Under natural conditions, trichomonads live in habitats rich in bacteria, so that it is not surprising to find that complete anaerobiosis provides the optimum condition for cultivation.

*Carbohydrate metabolism.* The utilisation of sugars varies appreciably between species, although only *T. gallinae*, *T. vaginalis* and *T. columbae* have been investigated in detail. Maltose, starch and glycogen appear to be the most efficient substrates, although glucose, fructose, galactose and sucrose are utilised to a lesser extent. A number of other carbohydrates are utilised to a varying degree, and there is some evidence of adaptation to these after several generations. The end products of metabolism are mainly fatty acids and are unusual in including hydrogen as well as carbon dioxide.

Attempts to demonstrate the tricarboxylic-acid cycle in *T. vaginalis* have produced somewhat conflicting results; evidence has been produced for the cycle in *T. gallinae* but not in *T. vaginalis*.

*Protein metabolism.* This has been little studied. The following amino acids have been found to be essential for the growth of *T. foetus*: arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine and valine.

*Fat metabolism.* There are no data available on this aspect of metabolism.

*Growth factors.* Axenic growth of trichomonads has only been obtained in complex media, so that it is difficult to obtain unequivocal data on growth requirements. Cholesterol (or closely related compounds), ascorbic acid, linoleic acid and panthothenic acid play important roles in the metabolism of various species of *Trichomonas*.

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## CHAPTER V

# HAEMOFLAGELLATES

The haemoflagellates are not confined in their distribution to the blood stream, but many species have adapted themselves to an intracellular existence and have invaded various tissues—especially those of the reticulo-endothelial (lymphoid-macrophage) system. This system plays an important role in the defence mechanism of the body and its essential components are the macrophages which are particularly abundant in the blood-forming and blood-destroying organs, the spleen, bone marrow, lymph glands and liver. Macrophages normally ingest and immobilise invaders of the blood stream and can similarly treat certain haemoflagellates. Many species, however, are resistant to the lytic action of these cells, and one particular group, the leishmanias, have made them their exclusive habitat. Thus, these remarkable organisms survive and reproduce in the very cells the body utilises to ingest invaders; their ability to survive in cells of the reticulo-endothelial system is shared with the exo-erythrocytic stages of malarial organisms.





Some species of organisms discussed under the heading of 'haemoflagellates' are intestinal parasites of invertebrates, but their relationships with the blood-dwelling forms are so close that it would be creating an artificial distinction to treat them separately, and hence they are considered here also.

### 5.1 General Account

*Occurrence.* Although the species most frequently studied are those which occur in mammals, particularly man and domestic animals, haemoflagellates occur in most vertebrates and many invertebrates. It is significant that, whereas those parasitic in vertebrates require an intermediate host (usually a blood-sucking insect but occasionally a leech), those parasitic in invertebrates can undergo their entire life cycle within the same host. This strongly suggests that the whole group was originally parasitic in the alimentary canal of insects.

*Morphology.* There are four basic morphological types found in the haemoflagellates,

TABLE 10  
STAGES IN THE LIFE CYCLE OF FIVE GENERA OF 'HAEMOFLAGELLATES'.  
(+ stage present; — stage absent)

Genus	STAGE			
	 Trypanosome	 Crithidial	 Leptomonad	 Leishmanial
<i>Leptomonas</i>	—	—	+	+
<i>Herpetomonas</i>	+*	+	+	+
<i>Crithidia</i>	—	+	+	+
<i>Trypanosoma</i>	+	+	+	+
<i>Leishmania</i>	—	—	+	+

\* Not identical with 'trypanosome' type.

and each of these to a greater or lesser degree can be transformed into the other (see Table 10). The conditions under which these morphological transformations occur are not understood and, indeed, little attempt has been made to elucidate them. It is known that change in temperature is one factor inducing transformation at least in *in vitro* cultures. Only the trypanosome type will be discussed in detail here, and from this type the others may readily be derived.

A typical trypanosome (Fig. 14) has an elongated flattened body, with a single nucleus containing a large central karyosome. Movement is effected by a single flagellum

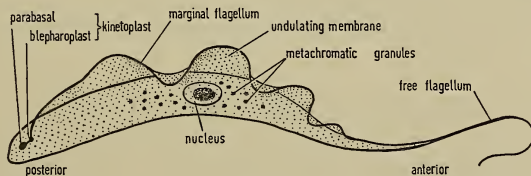


FIG. 14. A typical trypanosome; based on *T. gambiense* (after Chandler, 1955).

which arises at the posterior end of the body and runs along an undulating membrane to the other end, where it may either end or continue anteriorly as a *free* flagellum. The flagellum arises, as in intestinal forms, in a small basal granule or blepharoplast in front of which is a chromatin structure, the *parabasal*, the whole forming a complex, the *kinetoplast*.

The four basic morphological stages (Table 10) are as follows:

(a) *The leptomonad stage*: probably the most primitive. Body elongate and nucleus



central with the kinetoplast and starting point of the flagellum at extreme anterior end. No undulating membrane. All other types may be considered to have arisen from this type. Invertebrates only.

(b) *The crithidial stage*: kinetoplast has shifted back to a juxta-nuclear position and the flagellum is now connected to the body by an undulating membrane. Invertebrates; rarely in vertebrates.

(c) *The trypanosome stage*: as described above. The kinetoplast has moved to the posterior end of the organism and the flagellum is attached to the body for most of its length. Undulating membrane may be absent. Vertebrates and invertebrates.

(d) *The leishmanial stage*: body rounded and flagellum lacking. Any of the other stages may assume this form or conversely be developed from it. Vertebrates and invertebrates.

Of these four stages, only three occur in vertebrates, but any or all may occur in the alimentary canal of insects. When crithidial or leptomonad stages are detected in the intestine of an insect, it is thus difficult to determine whether they are merely insect parasites or whether they represent the developmental stages of a haemoflagellate of a vertebrate animal. In some cases, *crithidia*, believed to be true insect parasites, have been found to develop into fully developed trypanosomes when introduced into the blood of certain vertebrate hosts.

*Classification.* On the basis of the morphological types outlined above, haemoflagellates, which all belong to the family *Trypanosomidae*, are divisible into five genera, of which the first three are not considered in detail here. A sixth genus, *Phytomonas*, parasitic in plants, is also not considered.

### 5.2 Genera: *Leptomonas*, *Herpetomonas*, *Crithidia*

Genus *Leptomonas*. The leptomonads are exclusively parasites of invertebrates. The most easily obtainable form is probably *Leptomonas ctenocephali*, a species which lives in the intestine and malpighian tubules of the dog flea *Ctenocephalides canis*. It exists in the fore-gut as the leptomonad form, but in the hind-gut as the leishmanial stage. The former is slender and curved and up to  $18\mu$  long, the latter may be only  $3\mu$ . Multiplication is by binary fission.

Genus *Herpetomonas*. A leptomonad-like form but placed in a separate genus on account of the occurrence of a trypanosome stage in its life cycle; it can undergo transformation into any of the other stages. The most readily available species is *Herpetomonas muscaedomesticae* from the intestine of house flies. Transmission is by faecal ingestion.

Genus *Crithidia*. Exclusively parasites of invertebrates, particularly insects, but

many may represent stages in the development of vertebrate trypanosomes. The shape is long and slender. A common species is *Crithidia gerridis* in water bugs of the genera *Gerris* and *Microvelia*, where it occurs in the alimentary canal. It can transform into leptomonal and leishmanial stages.

### 5.3 Genus *Trypanosoma*

#### 5.31 General Account

Trypanosomes occur in the blood and some tissues of the majority of vertebrate animals, both warm- and cold-blooded. The life cycle involves an intermediate host, usually an insect, occasionally a leech, and in one species transmission is venereal. It is probably true to say that the great majority of trypanosomes are non-pathogenic and live at peace with their hosts, some of which can withstand heavy infections without developing any apparent symptoms.

Many of the best known species are, however, pathogenic, and the morphology and physiology of these have been most extensively studied. The disease produced by such forms is termed 'Trypanosomiasis'. One curious feature of some pathogenic species is that they may also be parasitic in other animals in which they are apparently harmless. In these natural hosts, the tissues have become physiologically adapted to the presence of the parasites through long periods of association, and such animals act as 'reservoir' hosts for these potentially pathogenic species.

*Morphology.* This has already been discussed on p. 51. Species diagnosis depends on differences in size and shape of the body, position of nucleus and degree of development of the undulating membrane and flagellum.

*Reproduction.* Multiplication is typically by binary fission, although in some species, due to factors not understood, cytoplasmic division is retarded without affecting nuclear division and a kind of multiple fission results.

*Life cycle.* Transmission from one vertebrate host to another is carried out by blood-sucking invertebrates, and in the case of mammals this is usually an insect, but in amphibian parasites (e.g. *T. inopinatum*) it is a leech. When the blood is drawn into the insect gut, after a short delay the trypanosomes become transformed into the crithidial forms, and these in turn give rise to trypanosomes differing slightly from the blood forms and known as *metacyclic trypanosomes*. This mode of transmission is known as *cyclical* to distinguish it from *mechanical* transmission, a process in which trypanosomes merely survive on and about the mouth parts of an insect for a short time, probably minutes, and are inoculated into a new host when the insect bites again, without undergoing any developmental cycle.

It is important to distinguish between these two types of transmission, because trypanosomes transmitted by the purely mechanical method can only be transmitted by bites which rapidly succeed one another, while those transmitted by the cyclical method cannot be transmitted until sufficient time has elapsed to enable them to reach an infective stage by a particular developmental sequence in the insect vector, a cycle usually requiring 15–35 days.

Cyclical development may culminate in two sites within the insect, the hind gut (technically referred to as the 'posterior station'), or the fore gut (the 'anterior station'), and this environmental discrimination has resulted in the occurrence of two methods of infection:

(a) *contaminative*: characteristic of hind-gut developing forms; vertebrate host infected by faeces (e.g. *T. cruzi*).

(b) *inoculative*: characteristic of fore-gut dwelling forms; metacyclic forms injected in saliva on biting new host (e.g. *T. rhodesiense*).

*Terminology.* In the classification of trypanosomes, the character of the flagellum often serves as a useful criterion and species are said to be *monomorphic* or *polymorphic*.

*Monomorphic*: if all the individual trypanosomes of the species possess a free flagellum, or alternatively, if none of the individuals possess a free flagellum.

*Polymorphic*: if some of the individuals of the species possess a free flagellum while others do not.

### 5.32 Type Example: *Trypanosoma lewisi*

definitive host:	rat
location:	blood stream
transmission:	contaminative, via <i>Nosopsyllus fasciatus</i>

*Occurrence.* The species is common in *wild* rats of the genus *Rattus* in all parts of the world. It is conveniently studied in the laboratory in the albino rat. It is a non-pathogenic species, often occurring in concentrations of 20,000–600,000 trypanosomes per cubic millimetre.

*Life cycle* (Fig. 15). During the first four or five days of an infection, reproduction—mainly by multiple fission—rapidly takes place. This gradually ceases, and on about the tenth day there is a 'crisis' when most of the parasites, which are now mainly long forms (the so-called 'adults'), are destroyed. The remaining trypanosomes continue to live for several weeks or months, until a second 'crisis' occurs when all (or practically all) the organisms are destroyed. Immunity is thus rapidly established. The laboratory maintenance of this organism, therefore, requires repeated passage through fresh rats at regular intervals; intraperitoneal injection being the most suitable method.

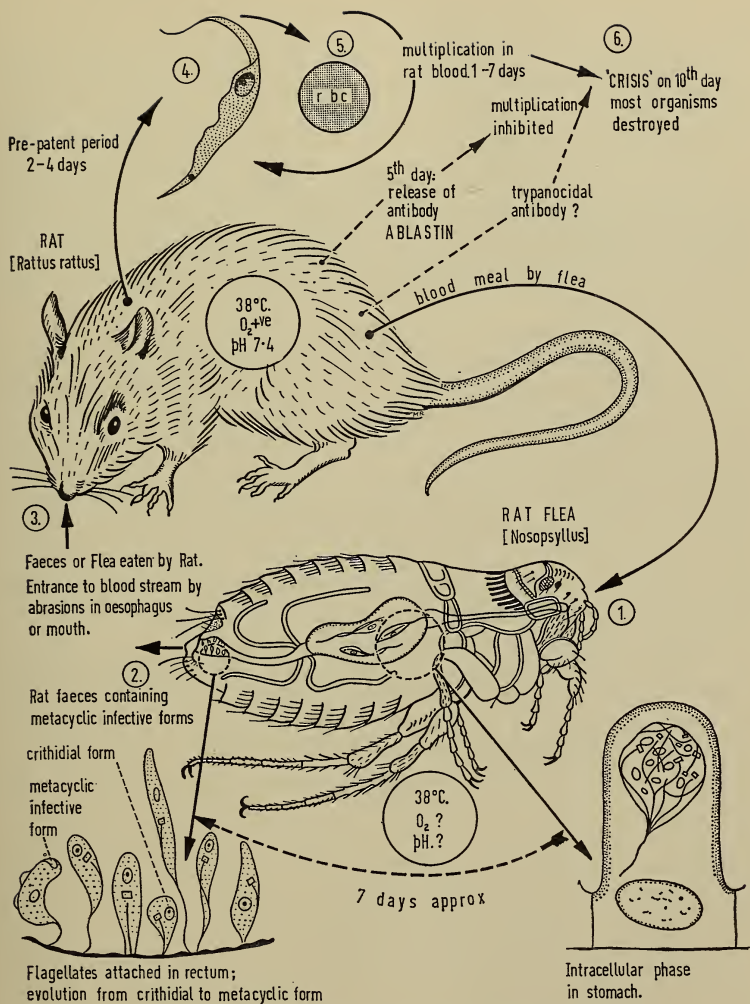


FIG. 15. Life cycle of *Trypanosoma lewisii*, a non-pathogenic haemoflagellate of the rat (partly after Hegner *et al.*, 1938).

Transmission is of the contaminative type and is carried out by ingestion of flea faeces or an entire flea; both these may occur when a rat licks its fur. The common rat flea of Europe and N. America, *Nosopsyllus fasciatus* is the usual host, but transmission can also be effected by *Pulex irritans* (of man) and *Xenopsylla cheopis*, the Indian rat flea.

When a flea makes a blood meal, the trypanosomes multiply in the mid-gut, finally invading the cells of the gut wall. Within these cells, rapid multiplication takes place and daughter trypanosomes are released to infect other cells. This process may be repeated several times. Eventually, the organisms make their way to the hind-gut and rectum and give rise to crithidial forms. The latter become transformed into stumpy metacyclic forms which constitute the infective stages discharged in the faeces.

*In vitro culture.* *T. lewisi* is readily cultured in any of the usual blood agar media used for trypanosomes (p. 401).

*Immunity.* The remarkable cessation of multiplication after about seven to ten days has been the subject of considerable research. The generally held view is that this is a response to an antibody termed *ablastin* (Taliaferro, 1932) which, while not being trypanocidal, prevents multiplication. The later crisis, which reduces the numbers and finally eradicates them is considered by some workers to be due to a trypanocidal antibody; an agglutinating antibody has also been postulated. An alternative view is that *ablastin* is indirectly responsible for both the inhibition and destruction of the trypanosomes. On this view, the appearance of *ablastin* results in agglutination, the agglutinated parasites being mechanically filtered out in the spleen, liver, etc., and destroyed by phagocytic action. This would account for the first number crisis. As the numbers became reduced opportunities for agglutination would decline and final elimination of the residual trypanosomes would be due to phagocytic action only.

Convincing evidence has been produced to suggest that *ablastin* is directed against metabolic products (see p. 381) secreted or excreted by the parasites (Thillet and Chandler, 1957, and Chandler, 1958). It has been shown that trypanosome-free metabolic products when injected into non-immune rats, after allowing ten days for the immunity to develop, result in development of complete protection against *T. lewisi*. It is thought that *ablastin* acts specifically against an enzyme or enzymes concerned in the nutrition of the parasites. Immunity in this organism is further discussed on p. 381. *Development in heterologous hosts.* *T. lewisi* exhibits a well-defined host specificity, developing optimally only in the rat. Behaviour in heterologous hosts has been conflicting. Survival, with little increase in population levels, has been reported in the guinea pig and the white mouse. In contrast, there have been many reports of failure to establish the organism in white mice, dogs, horses and cats. Lincicome (1958) has shown that by restricting the diet of white mice and using a supplement of rat serum, it is possible to obtain luxurious growth in this heterologous host. The reason for this



beneficial action of the serum supplement is unknown. It may be protective, it may block the reticulo-endothelial system, or it may supply growth factors unavailable in the heterologous host.

### 5.33 Classification of Trypanosomes

Trypanosomes are often considered as falling into four groups on grounds of morphology or behaviour. There is also evidence (p. 67) that marked physiological differences exist between these groups. Only selected examples marked thus \* are dealt with here. The characters are defined by Hoare (1949).

#### Brucei-Evansi Group

*T. brucei*\*

*T. rhodesiense*\*

*T. gambiense*\*

#### Vivax Group

*T. vivax*

*T. uniforme*

#### Congolense Group

*T. congolense*

*T. simiae*

#### Lewisi Group

*T. theileri*

*T. lewisi*\*

*T. cruzi*\*

### 5.34 Trypanosomes Infecting Man

There are two main types of trypanosomiasis of man caused by organisms in the *brucei-evansi* and *lewisi* groups.

Sleeping sickness: caused by *T. gambiense* and *T. rhodesiense*; occurs in tropical Africa.

Chagas' disease: caused by *T. cruzi*; occurs in Central and South America.

*T. gambiense* and *T. rhodesiense*

The organisms *T. gambiense* and *T. rhodesiense* in man are morphologically indistinguishable from *T. brucei*, which is a natural parasite of wild game in Africa, particularly antelopes. The latter mammal represents the natural reservoir host of this trypanosome in which it is non-pathogenic. There is no simple criterion whereby these three species can be distinguished; the only satisfactory method is to test their effect on man or laboratory animals. Each can thus be regarded, not as distinct species (although it is clearly convenient to regard them as such), but as biological races of the same species adapted to different hosts.

**Morphology.** Characterised by marked polymorphism manifested by the presence of three main types of blood forms:

(a) *slender forms* (Fig. 16): long and thin, about  $29\mu$  long, free flagellum.

(b) *stumpy forms* (Fig. 16): stout and short, average length  $18\mu$ , typically no free flagellum, but a short one may be present.

(c) *intermediate forms* (Fig. 16): about  $23\mu$  long with a medium thick body and a free flagellum of medium length.

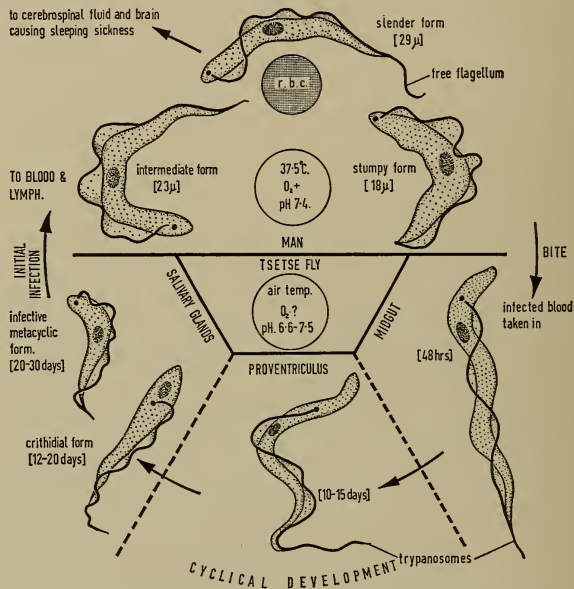


FIG. 16. Life cycle of *Trypanosoma gambiense* or *T. rhodesiense* (modified from Hoare, 1949).

In a typical infection, the proportion of trypanosomes belonging to any of these three types can vary within considerable limits, although slender forms usually predominate. Polymorphism is lost after passage in laboratory animals for some time, and the organisms become monomorphic.

**Course of infection.** *T. gambiense* and *T. rhodesiense* live in the blood, lymph glands and



ducts for the first two weeks or so of the infection, undergoing extensive multiplication. During this time, as a protective measure, there is local proliferation of the phagocytes, resulting in enlargement of the lymph glands. The active forms in the blood may disappear due to destruction by trypanolytic antibodies, but reappear later as the more resistant forms multiply. The periodic disappearance of the trypanosomes is called 'the crisis'.

After about a month (*T. rhodesiense*) or more (*T. gambiense*), the organisms invade the cerebro-spinal fluid and later the brain may become involved. The resulting 'sleeping sickness' is characterised by a general physical and mental depression and a desire to sleep. In untreated cases of the Gambian form, the disease may last for several years before ending in death, but in the Rhodesian form, it runs an acute course lasting about six months, and also terminates fatally.

*Infection in other mammals.* In addition to man, these species are infective to numerous other mammals, some of which occur in the endemic area.

*Transmission.* The vector of sleeping sickness is the tsetse-fly, a name derived from the native name for flies of the genus *Glossina*. Although it is possible for the trypanosomes to develop in many species of *Glossina* under normal conditions, only a few species act as natural carriers, thus:

*T. gambiense*—*G. palpalis*, *G. tachinoides*.

*T. rhodesiense*—*G. morsitans*, *G. swynnertoni*, *G. pallidipes*.

Both males and females are blood-sucking and act as carriers.

*Cyclical development in Glossina.* Ingested parasites multiply rapidly in the mid intestine, for a period of 10–15 days, after which they begin to give rise to slender proventricular forms which become concentrated in the proventricular region. These make their way into the salivary glands (via a soft spot in the peritrophic membrane). Here they attach themselves to the walls by means of their flagella or lie free in the lumen, developing into crithidial forms. These undergo further multiplication and give rise to small forms, with or without a short flagellum and somewhat resembling the stumpy blood forms; these are the infective metacyclic trypanosomes. Normal cyclical development takes 20–30 days at 75–85° F. Many flies seem resistant to infection, and in nature only about 1–2 per 1,000 wild flies are found to be infected.

*Reservoir hosts.* In addition to man, several other animals may act as reservoirs for the trypanosomes of sleeping sickness. In antelopes, for example, infections with *T. gambiense* and *T. rhodesiense* may last two years and up to four years in other animals (pigs, dogs, goats) without apparently loss of transmissibility to man. Other animals, especially game animals, such as buffaloes, elephants and warthogs have a high rate of

infection with trypanosomes of the *brucei* group, and, as emphasised earlier, since *brucei*, *gambiense* and *rhodesiense* are morphologically indistinguishable, it is difficult to obtain precise information as to which animals are acting as reservoirs. It seems likely that in endemic areas, the trypanosomes of game animals may be any of these three species, whereas in non-endemic areas, they are probably only *T. brucei*.

*Prevention.* This involves as usual, breaking the cycle of the parasite at some stage, either by treating patients with drugs, disposing of the flies or reducing contacts between them and the human population. Intelligent use of insecticides and determined attacks on tsetse-fly breeding areas, as well as destruction of reservoir game animals on a large scale, are indicated.

### *Trypanosoma cruzi*

This organism (also referred to as *Schizotrypanum cruzi*) causes Chagas' disease or American human trypanosomiasis, which may be fatal. It occurs throughout South and Central America, especially Brazil, Argentine and Mexico.

*Morphology.* A monomorphic form, about  $20\mu$  in length, and characteristically curved (Fig. 17). The kinetoplast is large, considerably larger than in any of the species discussed already, and sometimes appears as a bulge at the posterior end. The flagellum is of medium length.



FIG. 17. *Trypanosoma cruzi*, from experimentally infected mice (after Brumpt, 1949).

*Multiplication.* This species is unlike any of those already studied, in that division does not take place in the peripheral blood (Fig. 18). The trypanosomes disappear periodically from the blood stream and enter certain tissues, especially those of the reticulo-endothelial system and the heart. They show marked predilection for the heart in which they penetrate the muscle fibres, lose their flagella and assume the leishmanial form (Fig. 19). The latter multiply by binary fission and ultimately give rise to crithidial forms; these undergo binary fission and give rise to trypanosomes which appear in the peripheral blood. The cycle is repeated con-

tinuously so that the trypanosomes are constantly appearing and disappearing from the blood. Periodical disappearance from the blood stream may not occur in experimental animals.

*Transmission.* The vectors of *T. cruzi* are the brightly coloured bugs of the family Reduviidae, all stages of which (larva, nymph and imago) are susceptible to infection. The most important natural vectors are *Panstrongylus megistus*, *Triatoma infestans* and *Rhodnius prolixus*. Although triatomids are probably the natural hosts, the organism is

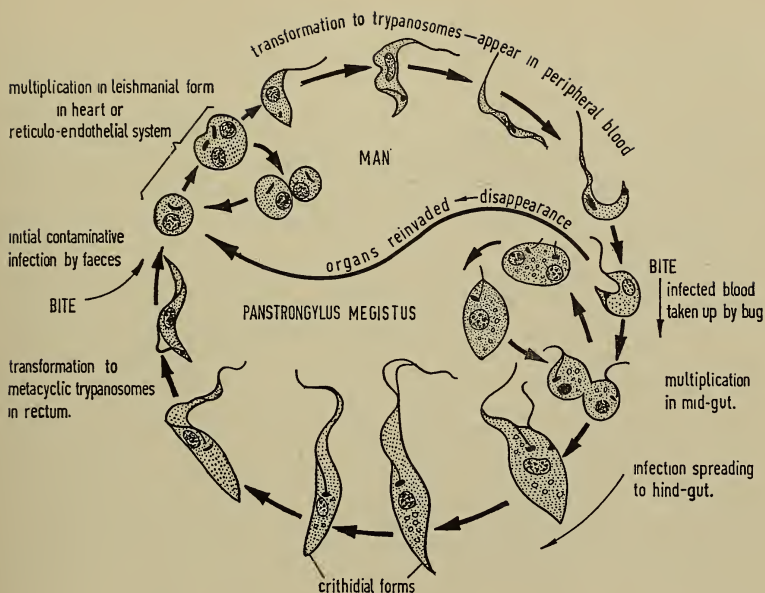


FIG. 18. Life cycle of *Trypanosoma cruzi* in man and the triatomid bug *Panstrongylus* (= *Triatoma*) *megistus*; other arthropods, bed bugs, ticks and keds can also act as vectors (after Brumpt, 1949).

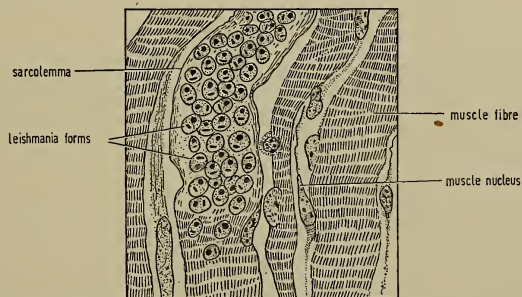


FIG. 19. *Trypanosoma cruzi* within the muscle fibres of a rat (after Brumpt, 1949).

not particular about its vectors and will develop in other arthropods, especially bed bugs and ticks.

*Development in vector.* Transmission is contaminative and development in the vector culminates in the 'posterior station'. In the mid-gut, the trypanosomes develop into crithidia and multiply rapidly, eventually passing to the rectum. Here they give rise to metacyclic infective trypanosomes which are voided with the faeces. Infection comes about by rubbing a bite-wound contaminated by faeces. The eyelids are a favourite site for infection.

*Other hosts.* Unlike many trypanosomes, *T. cruzi* has a wide host spectrum and some forty different species of animals in the New World have been found to be infected naturally. The most important host reservoirs are *Dasyppus* (armadillo) in S. America; *Didelphis* (opossum) in S. America, Panama and the U.S.A.; dogs, pigs and cats in S. America. Rats, cats, rabbits or mice are suitable laboratory hosts.

*Human infection.* Infections occur most frequently in infants and children; in adults there seems to be a high natural resistance, but this may be greatly weakened by malnutrition. The eye is the commonest site of infection, and the symptoms, swellings and inflammation, appear in this region first. The initial swelling is known as 'chagoma'. After multiplying for some days in the subcutaneous tissues, the leishmanial forms give rise to trypanosomes which in turn invade other organs and tissues. As emphasised earlier, the heart is commonly attacked. Severe cases may end in death.

If direct examination of the blood fails to reveal the trypanosomes in suspected Chagas' disease, the infection can be detected by allowing some *reduviid* bugs to feed on the patient. In positive cases, the developmental forms of the droppings are found in the droppings seven to ten days later.

## 5.4 Genus *Leishmania*

### 5.4I General Account

The flagellates of the genus *Leishmania* are mainly parasites of man, dogs and some other mammals, and cause diseases collectively known as *leishmaniasis*, which may be divided into two groups.

(a) Kala-azar or *visceral leishmaniasis*.

(b) Oriental sore or *cutaneous leishmaniasis*. A variety of this type is *Espundia* or *muco-cutaneous leishmaniasis*.

*Morphology.* In man, the parasites occur in the leishmania form only (Fig. 20), but the leptomonad form is assumed in the insect vector. The parasites are small, ovoid or round bodies (often called Leishman-Donovan bodies) about  $2-5\ \mu$  in diameter, con-

taining two structures readily seen in preparations stained by one of the Romanovsky methods. These are the nucleus and the kinetoplast, the latter being either granular or rod-like.

No morphological differences have been found between the species of man and other animals, but solely on the grounds of clinical differences, geographical distribution and different serological reactions, the same morphological organism has been given four different specific names: *Leishmania donovani*, *L. infantum*, *L. tropica*, *L. braziliensis*. These may be regarded as biological races of two species, *L. donovani* and *L. tropica*.

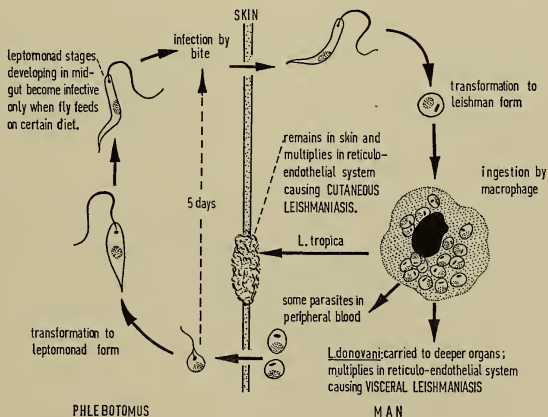


FIG. 20. Life cycle of *Leishmania donovani* and *L. tropica* in man and vector, *Phlebotomus argentipes* (partly after Hoare, 1949).

**Habitat and life cycle.** Leishmanias are unusual in living entirely within the cells of the reticulo-endothelial system to which they appear to have become perfectly adapted, since the proteolytic enzymes which attack other invaders of the blood stream do not destroy them. The life cycle is shown in Fig. 20. Within the macrophages, the parasites multiply by binary fission at an estimated rate of once in every 24 hours (Stauber, 1955). The later stages of development in the reticulo-endothelial system are not properly understood. In experimentally infected hamsters, the parasites accumulate rapidly in the spleen and liver, especially the latter. After about seven to eight days, the rate of accumulation decreases sharply in the liver, suggesting acquired host resistance. This effect may be a result of the cessation of the reproductive activity in the originally in-



fected host cells due to their destruction and the release of parasites into the blood stream. On entering the blood stream, the parasites would then be in contact with possible destructive forces for the first time (Stauber, 1955).

*Transmission.* Like other examples in parasitology, several vectors were erroneously incriminated before it was established that the true vectors are sandflies of the genus *Phlebotomus*, a fact only firmly established in 1942.

In 1907, it was found that the organisms could undergo development in the bed bug *Cimex lectularius*, and it was assumed that this was the vector. But under no circumstances could the bed bug be made to transmit the disease to man.

It was later noted (1924) that the distribution of Kala-azar in India coincided rather closely with that of the sandfly *Phlebotomus argentipes*, and that flies became heavily infected with leishmanias after feeding on Kala-azar patients. Attempts at experimental transmission to hamsters and human volunteers failed, and it looked as though the bed-bug fiasco was about to be repeated once more. This, in fact, proved not to be the case, for some fifteen years later it was found that, if after taking in infected blood, sandflies were fed on raisins, the flagellates multiplied prodigiously within the flies and became infective to hamsters and man. The word 'fruitless' as applied to the early research can seldom have been so apt!

The specific factor required to stimulate production of infective forms has not been determined. Ascorbic acid is known to be one of the essential growth factors of *Leishmania* and it may be that this vitamin was lacking in previous diets of the flies.

The morphological changes within the sandfly gut are simple. In the mid gut they become leptomonad flagellates which multiply rapidly, spreading forwards to enter the oesophagus and pharynx by the fourth or fifth day. When introduced into the mammalian skin by a bite, the flagellates become rounded and assume the leishmanial form.

### 5.42 Particular Species of *Leishmania*

*Course of infection* (Fig. 20). The extent to which the organisms spread in the body governs the course of the infection. Where they remain localised in the skin, the *cutaneous* form of the disease results, which may be mild or severe. When the visceral part of the reticulo-endothelial system becomes infected and the spleen, liver and bone marrow become involved, the more serious *visceral* leishmaniasis develops and death may result in untreated cases. Alteration in the serum proteins in visceral leishmaniasis in man and animals is readily demonstrable electrophoretically, and is characterised by a fall in serum albumen and a rise in globulin in the later phases of the infection.

Although there are only two recognised species of *Leishmania*, *L. donovani* and *L. tropica*, there are several biological races of these species, and for convenience the parasites may be listed under the following specific names which are in general usage, depending on the distribution of the diseases:

*L. donovani*—causes Kala-azar in Assam and India.

*L. infantum*—causes infantile Kala-azar in children in Mediterranean area.

*L. tropica*—causes Oriental sore (Delhi boil or Aleppo button) in Mesopotamia and Near East.

*L. braziliensis*—causes Espundia in Central and South America.

*L. donovani*. This is a visceral species with a predilection for the spleen, liver and bone marrow, which may produce a generalised infection of the reticulo-endothelial system. The main effects of the disease are due to the blockage of the reticulo-endothelial system, anaemia resulting from the invasion of bone marrow, and intoxication due to release of metabolic by-products by the parasites. A cutaneous infection may appear at a later stage. Untreated cases are usually fatal. Immunity appears to be readily developed. *L. donovani* may be easily transmitted to cotton rats and golden hamsters which make excellent laboratory hosts.

*L. infantum*. Causes the infantile form of Kala-azar in the Mediterranean region; it affects mainly children under the age of five. It is probably closely related to a visceral leishmaniasis which occurs in dogs. In areas where wholesale destruction of dogs has been carried out, there has been a reduction of the incidence in children. The disease is, therefore, probably a *zoonosis* (i.e. produced by parasites of animals).

*L. tropica*. This organism causes cutaneous leishmaniasis (Oriental sore, Delhi boil, Aleppo button) characterised by a local invasion of the reticulo-endothelial cells of the skin resulting in sores and ulcers. There is evidence again that this disease may also be a *zoonosis*, as a similar type occurs in dogs in the Old and New World, although the evidence is not entirely conclusive. Except for temporary development of a sore, the pathological effects are slight.

*L. braziliensis*. This race causes a type of mucocutaneous leishmaniasis usually involving the mucous membrane of the mouth, nose and throat, which may become chronically ulcerated. Severe infections produce revolting distortions of the facial regions, and in untreated cases secondary infection, which may prove fatal, develops. This organism is confined to Central and South America.

### 5.5 Physiology of Haemoflagellates

Knowledge in this field has been reviewed by von Brand (1951, 1952), and Hutner and Provasoli (1955).

*Respiration*. The rate of oxygen consumption of the blood stream forms of trypanosomes is relatively high, but that of tissue and culture forms much lower. Blood-stream forms of *T. gambiense*, for example, consume  $170 \text{ mm}^3/10^8$  organisms/1 hr., whereas cultured forms consume only  $14 \text{ mm}^3/10^8$  organisms/1 hr. It is difficult to obtain accurate figures



for the consumption in terms of tissue weight on account of technical difficulties in obtaining reliable dry-weight estimations. It has been calculated that *T. rhodesiense* consumes 28.5 c.c O<sub>2</sub>/gm dry weight/1 hr., a figure which is interesting to compare with that of a resting young rat which uses only about 1.5 c.c O<sub>2</sub>/gm dry weight/1 hr. On the basis of relative surface, however, trypanosomes use some 400 times less O<sub>2</sub> per unit surface than a rat. These figures emphasise the difficulties of finding a common denominator for metabolic studies in animals widely separated in the animal kingdom, a point stressed by von Brand.

Many of the physical and chemical characteristics of the culture medium have a marked effect on the oxygen consumption. Ionic constitution and O<sub>2</sub> tension have little effect, but temperature and availability of sugar are extremely important, the temperature effect approximately following Arrhenius' equation. The presence of glucose is the most important single factor effecting oxygen consumption. As soon as the sugar in the medium becomes depleted, the respiratory rate declines, eventually becoming negligible. The age of the metabolising organisms may also be of importance, and the young dividing stages of *T. lewisi* show a lower oxygen consumption than that of older undividing forms. This result may be related to the presence of the antibody ablastin (p. 56) which appears in the early days of an infection and probably interferes with the oxidative glucose metabolism.

TABLE II

## RESPIRATORY QUOTIENT OF SOME MAMMALIAN TRYPANOSOMES.

sugar was available in all cases

(data from von Brand, 1952; Agosin and von Brand, 1954).

Species	Form	Average R.Q.
<i>Trypanosoma lewisi</i>	Blood stream (young)	0.74
<i>Trypanosoma lewisi</i>	Blood stream (old)	0.91
<i>Trypanosoma cruzi</i>	Culture	0.7
<i>Trypanosoma congolense</i>	Blood stream	1.0
<i>Trypanosoma equiperdum</i>	Blood stream	0.06
<i>Trypanosoma rhodesiense</i>	Blood stream	0.16
<i>Leishmania tropica</i>	Culture	0.95

*Respiratory quotient.* As in many other organisms, the R.Q. level is related to the availability of sugar in the medium. If available, the R.Q. is high; if lacking, the R.Q. is lower. Although the R.Q. in many instances is 1.0, it does not necessarily follow that a carbohydrate metabolism of a completely oxidative type is indicated. Trypanosomes are aerobic fermenters; that is, the sugar consumed is not oxidised completely—a

phenomenon not understood. Very marked differences exist between different species. The R.Q. of *T. lewisi* is high, whereas that of *T. rhodesiense* and *T. equiperdum* is extremely low (Table 11). It is clear that these differences arise from differences in glucose utilisation. In *T. equiperdum*, the degradation of glucose stops at the pyruvate stage and carbon dioxide is not evolved. On the other hand, in *T. lewisi*, degradation proceeds further in the glycolytic chain, and carbon dioxide is produced.

The R.Q. of *T. congolense* is also high (1.0), thus resembling *T. lewisi*, but in other aspects of its metabolism (see below) this species is somewhat intermediate between that of *T. lewisi* and that of trypanosomes of the *brucei-evansi* groups, a finding which fits in well with Hoare's classification of mammalian trypanosomes (p. 57).

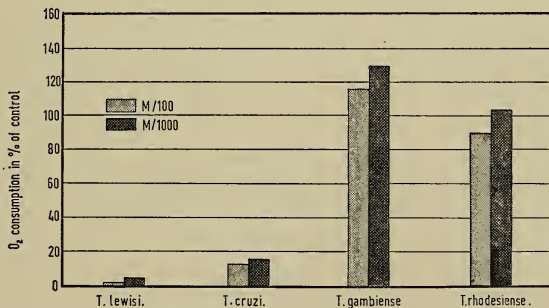


FIG. 21. Inhibition of O<sub>2</sub> consumption by KCN at concentrations of M/100 and M/1000. Note that the blood-dwelling forms *T. gambiense* and *T. rhodesiense* are unaffected (after von Brand, 1951).

*Respiratory enzyme systems.* Respiration of leptomonads, leishmanias and trypanosomes of the *lewisi* group is strongly inhibited by cyanide, whereas that of trypanosomes of the *brucei-evansi* group is not inhibited but slightly stimulated (Fig. 21). This demonstrates that the former groups of organisms contain enzyme systems depending for their activity on heavy metals, probably iron, and hence the cytochrome system may be important. Inhibitors of cytochrome oxidase, such as azide or H<sub>2</sub>S, inhibit respiration also, a result compatible with the presence of cytochrome oxidase.

The failure of cyanide to inhibit respiration in the *brucei-evansi* group suggests that either these organisms do not possess metal-dependent enzyme systems or that they have available alternative pathways for use in the event of blockage of the normal metabolic routes.

In the absence of a heavy metal system, it must be concluded that the transfer of hydrogen must be carried out by dehydrogenase systems. Application of the Thunberg technique with known inhibitors (i.e. iodoacetic acid) has shown such systems to be present, although no definite system has been identified. The dehydrogenase systems commonly associated with respiration—succinic dehydrogenase, coenzyme I oxidation-reduction—or the malic dehydrogenase systems appear to be absent.

In view of the intermediate position of *T. congolense* referred to above (p. 67) it is of interest to note that this organism is apparently intermediate between the *brucei-evansi* group and the *lewisi* group in its sensitivity to cyanide (M/1000 gives 40 per cent inhibition). (Agosin and von Brand, 1954.)

*Carbohydrate metabolism.* As glucose is the sugar normally available in the blood stream, it is not surprising to find that most trypanosomes metabolise it. An exception is *T. cruzi*, for which a glucose consumption of the blood-stream form has never been demonstrated; the cultured form can, however, use glucose. The hexoses, fructose and mannose are also good sources of energy, but galactose is utilised to a limited degree only by some

TABLE 12  
RELATIVE UTILISATION RATES OF CARBOHYDRATES BY  
HAEMOFLAGELLATES IN AXENIC CULTURE

(data from von Brand, 1952)

Species	Form	Glucose	Mannose	Fructose	Galactose	Maltose
<i>Trypanosoma lewisi</i>	blood	100	132	50	0	218[?]
<i>Trypanosoma cruzi</i>	culture	100	—	96	—	3
<i>Trypanosoma brucei</i>	blood	100	86	21	9	50
<i>Leishmania donovani</i>	culture	100	69	77	31	5
<i>Leishmania tropica</i>	culture	100	—	88	—	2

species (Table 12). Maltose and glycerol are also good substrates. The carbohydrate requirements of tissue and blood-dwelling forms may vary considerably. The concentration of glucose in the medium is of minor importance as trypanosomes appear to be able to extract sufficient quantities of glucose even from dilute solutions. This would clearly be advantageous when living in a glucose-low environment such as the cerebrospinal fluid in which glucose concentration is less than that of blood (Table 6).

*End products of carbohydrate metabolism.* The incomplete oxidation of glucose and other carbohydrates is a characteristic of the trypanosomes. As figures given for the R.Q. (p. 67) indicate, CO<sub>2</sub> is produced by all culture forms studied and by *T. lewisi*, but the blood stream forms of other species produce little (*T. rhodesiense*) or practically none

(*T. equiperdum*). The end products other than  $\text{CO}_2$  are mainly fatty acids, the nature of which varies in different species, showing that degradation does not proceed equally far along the glycolytic pathway. The production of such acids is demonstrable either by a pH drop during incubation or by evolution of  $\text{CO}_2$  from bicarbonates in the medium. The following end products have been detected: pyruvic, lactic, formic, acetic, oxalic, and succinic acids, ethyl alcohol, glycerol. Pyruvic, lactic, formic and succinic acids are most commonly produced.

*Intermediary carbohydrate metabolism.* The picture of the intermediary carbohydrate metabolism in trypanosomes is very incomplete and shows wide variation in different species. The evidence suggests that the initial steps in the anaerobic degradation follow the familiar Embden-Meyerhof sequence (Table 38), but that deviations are possible. Some glycolytic enzymes have been identified (e.g. aldolase) and the occurrence of phosphorylation in several species has been definitely established. On the other hand, the ability of trypanosomes to oxidise substrates other than carbohydrate is in favour of the possibility of a non-glycolytic dissimilation.

*Fat metabolism.* There is no evidence that trypanosomes can derive energy from triglycerides of fatty acids or from lipid in general. Lipases have not been detected.

*Protein metabolism.* Trypanosomes can apparently utilise proteins for energy purposes probably by deamination. The energy derived, however, is not sufficient to sustain them and in the absence of carbohydrate they rapidly die. The protein-splitting enzymes of trypanosomes have been little studied. A cathepsin-like protease, a carboxy-polypeptidase, an amino-polypeptidase and dipeptidase have been detected in *T. evansi*, but not pepsin- or trypsin-like enzymes. How far these enzymes can break down serum protein is not known, and protein required for protein synthesis may be derived from lower proteins or free amino acids.

*Growth factors.* Trypanosomes or leishmanias cannot be grown in culture in the complete absence of haemoglobin whether free or contained within erythrocytes. It is known that one of the indispensable constituents contained in blood is haematin, an iron salt of protoporphyrin. The role of haematin in the metabolism is unknown, but it is presumably related to the presence of an iron-catalysed enzyme system. A further known factor is ascorbic acid, normally present in erythrocytes. Its role could be merely that of lowering the oxidation-reduction potential of the culture or of destroying certain toxic oxidation products formed in the media. If this were so, however, other reducing agents could replace it and this has not been found to be the case.

*In vitro culture.* Most Trypanosomidae of insects may be readily cultured *in vitro* (p. 401) as well as those of the *lewisi* group. Members of the *brucei-evansi* group are more

difficult to culture, but fluid, semi-solid and diphasic blood-containing media have been used with some success. In all cultures of two-host trypanosomes, the cultured forms *develop only to the stage reached in the gut of the intermediate host*, and to date it has not been possible to cultivate any trypanosome to a stage normally assumed in the blood stream.

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## CHAPTER VI

### SPOROZOA:

### GREGARINES AND COCCIDEANS

Unlike the groups of Protozoa already studied, the Sporozoa lack organs of locomotion. The majority are intracellular parasites depending for nourishment on soluble cellular materials and unable to ingest particulate food or bacteria. In vertebrates, they occur mainly in the blood stream, the reticulo-endothelial system, and the mucous lining of the intestine; in invertebrates, they are found chiefly in the gut, digestive or excretory systems. Members of one sub-order, however, are intercellular parasites, and these have either organs of attachment or can move by an amoeboid-like movement. For transmission from host to host, sporozoans depend either on the production of highly resistant spores (which give the group its name) or make use of vectors, usually arthropods. The physiology of the group has been little studied.

#### 6.1 Classification (after Kudo, 1954)

##### **Class Sporozoa.**

*Sub-class Teleosporidea.* Intracellular during part of the life cycle; spores simple without polar filament.

*Order 1. Gregarinida.* Typically parasites of the digestive tract, body cavities or reproductive systems of invertebrates. Trophozoites usually free and form gametes directly or after several schizogonic cycles, e.g. *Monocystis agilis*.

*Order 2. Coccidia.* Typically intracellular parasites of the alimentary canal and associated glands of vertebrates and invertebrates. Multiplication by repeated schizogony. Eventually dissimilar gametes form which undergo syngamy; the zygotes are non-motile and become enclosed in spores. Alternation of generations between arthropod and molluscan hosts occurs in some species, e.g. *Eimeria stiedae*.

*Order 3. Haemosporidia.* Schizogony in fixed tissue cells, usually with subsequent



schizogony in erythrocytes. Gametocytes in vertebrate host with fertilisation in an arthropod vector; zygote motile and sporozoites naked, e.g. *Plasmodium berghei*.

## 6.2 General Biology

### 6.2I Order 1. Gregarinida

These are exclusively parasites of the alimentary canal and body or visceral cavities of invertebrates, usually arthropods or annelids, but occasionally tunicates. This group contains an enormous number of species, as almost any arthropod or annelid is parasitised by at least one species. The majority multiply only by sporogony, but a few undergo schizogony and on this basis they have been further sub-divided as follows:

*Sub-order 1. Eugregarinina.* No schizogony.

*Sub-order 2. Schizogregarinina.* Schizogony.

*Sub-order Eugregarinina. General account.* This order includes the majority of the so-called 'gregarines', which are common parasites of arthropods and annelids (Table 13). The best-known genera are *Monocystis* (often studied in elementary zoology classes), species

TABLE 13  
SOME REPRESENTATIVE GREGARINES

Species	Hosts	Site
<i>Monocystis agilis</i> . . .	<i>Lumbricus</i>	seminal vesicles
<i>Monocystis magna</i> . . .	<i>Lumbricus</i>	seminal vesicles
<i>Gregarina cuneata</i> . . .	<i>Tenebrio</i> (larva)	intestine
<i>Gregarina polymorpha</i> . . .	<i>Tenebrio</i> (larva)	intestine
<i>Gregarina steini</i> . . .	<i>Tenebrio</i> (larva)	intestine
<i>Gregarina blattarum</i> . . .	cockroaches	mid gut
<i>Gregarina rigida</i> . . .	<i>Melanoplus</i>	intestine
<i>Porospora portunidarum</i> . . .	crabs/molluscs	intestine/gills
<i>Nematopsis ostrearum</i> . . .	crabs/ <i>Ostrea</i>	intestine/gills
<i>Gonospora minchini</i> . . .	<i>Arenicola</i>	coelom
<i>Ophryocystis mesnili</i> . . .	<i>Tenebrio</i> (adult)	Malpighian tubules

of which occur in the seminal vesicles of earthworms, and *Gregarina* from the gut of mealworm larvae (*Tenebrio molitor*). The structure and life cycle of most species do not differ widely, except for variations in the structure used for attachment to the cell walls.

Typically, the organisms penetrate host cells and grow at their expense. They soon leave the cells but may become attached to them by various organallae of attachment. Later, the trophozoites become detached from the host cells and lie free in the lumen



of the organ. At this stage, they become vermiform, with ectoplasm and endoplasm clearly demarcated, and can move by an undulating movement.

In some species (the acephaline gregarines), the body is a single undivided unit (e.g. *Monocystis agilis*) but in others it is clearly partitioned into a smaller anterior part, the *protomerite*, and a larger posterior part, the *deutomerite*, which contains a single nucleus (Fig. 22). In some forms, the protomerite is drawn out into a specialised region for attachment known as the *epimerite*, which may be highly developed. Many gregarines are solitary but may move about in permanent end-to-end association with one another, a process called *syzygy* (Fig. 22). Trophozoites encyst in pairs, the nucleus

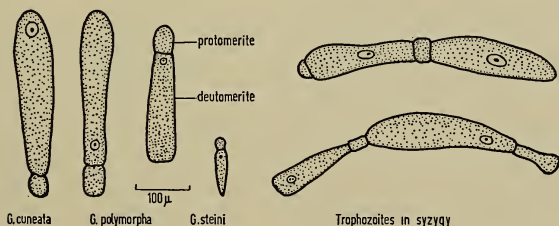


FIG. 22. Gregarines from gut of larval *Tenebrio molitor* (original).

in each individual breaking up into a large number of smaller nuclei which enter into the budding cytoplasm and form either isogamous (the same kind) or anisogamous (different kind) gametes. Each of the gametes from one individual (often called a gametocyte at this stage) conjugates with a gamete from the other and zygotes are formed. The zygotes develop a resistant spore coat and its contents divide several times to form eight sporonts.

*Modifications of the typical gregarine life cycle.* Some gregarines have two hosts in their life cycle, usually an arthropod and a mollusc. A typical example is *Porospora portunidarum* which has an alternation of generations between crabs of the genus *Portunus* and the mollusc *Cardium edule*.

The thick-walled oocysts of *Porospora portunidarum* contain only one sporozoite and when an infected mollusc is eaten by a crab, the sporozoite escapes through a micropyle into the intestine. The escaped sporozoites readily absorb nourishment and grow in size, becoming attached to the intestinal epithelium and eventually develop into a typically large gregarine with definite protomerite and deutomerite. Two or more trophozoites usually associate and become encysted in a gametocyst, but even a single gregarine can also encyst without a partner! By repeated divisions an enormous number of multinucleated bodies (gymnospores) are formed. Some workers identify this process as schizogony and include the species in

the Schizogregarinina. The gymnosporous are passed out into the faeces of the crab and if they reach the molluscan host are taken by phagocytes into the gills, mantle or digestive system. It is presumed that they break up into gametes, which conjugate to form the zygote which becomes the thick-walled oocyst. Release and conjugation of gametes does not appear to take place in the crab.

*Sub-order Schizogregarinina.* The schizogregarines are intestinal parasites of arthropods, annelids and tunicates. In general, they have life cycles similar to the eugregarines but also have a cycle of schizogony. *Schizocystis*, from the gut of the larvae of midges of the genus *Ceratopogen*, and *Ophryocystis*, from the Malpighian tubules of *Tenebrio* are readily obtainable genera.

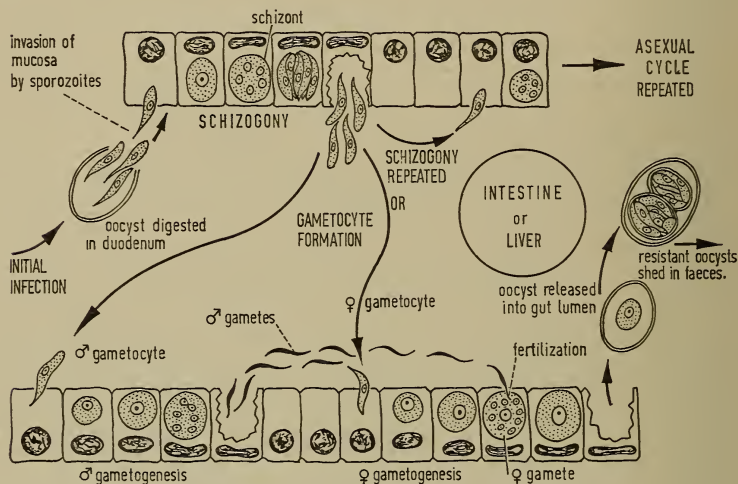


FIG. 23. Life cycle of a coccidian (based partly on Hoare, 1949).

## 6.22 Order 2. Coccidia

Coccidians attack a wide range of invertebrate and vertebrate hosts. The majority are parasites of the alimentary tract and its associated glands. Asexual reproduction takes place by schizogony and sexual reproduction by anisogamy with the production of highly resistant spores. Many species are pathogenic.

*Life cycle of a typical coccidian* (Fig. 23). The life cycles of only a few species have been worked out in detail, but since all known cycles are similar, it is likely that those which

have not yet been worked out will follow the general pattern. A typical life cycle consists essentially of several asexual generations (schizogony) followed by a sexual generation, generally ending in the development of an encysted stage or *oocyst* which is passed in the faeces.

In a typical cycle, a sporozoite enters an epithelial cell and becomes rounded to form a trophozoite. This gradually increases in size and undergoes schizogony in the course of which the nucleus of the organism (now a *schizont*) divides by a rapid succession of mitoses, so rapid in fact that a new spindle may be formed before preceding ones are completed, thus creating an impression of multiple nuclear division. When schizogony is completed the merozoites are released and these enter fresh cells and the cycle is repeated. The asexual cycle may continue for several generations, but eventually, under the influence of a stimulus believed to be associated with antibody formation by the host, some of the merozoites become differentiated into male and female forms which initiate the sexual cycle of development.

The sexual forms of the merozoites enter cells, become rounded, and differentiate into microgametocytes and macrogametocytes. The microgametocytes grow and undergo nuclear division and produce a number of comma-like microgametes which escape into the gut lumen. Meanwhile, each macrogamete has increased in size, and its nucleus has undergone reduction division. The cytoplasm of the macrogamete is characterised by possessing refractive globules around its edges. A microgamete penetrates a macrogamete and forms a zygote around which is developed a highly resistant spore case produced by the fusion of the refractive granules. Part of this spore case, at least, is a 'tanned protein' (Monné and Hönig, 1954) similar to the egg-shell of trematodes (p. 144), but it is more resistant, being one of the most resistant organic materials known. Oocysts may remain viable even after treatment with 5 per cent dichromate or 1 per cent chromic acid.

The encysted zygote, now known as an oocyst, is passed with the faeces. Outside the host body, it develops by sporogony, a zygote dividing into two sporoblasts each of which forms a cyst of its own, a sporocyst. Within each sporocyst, the organism divides twice to form four sporozoites, and the oocyst is now infective.

Almost complete immunity is usually developed if the host can recover from the primary infection.

Fig. 24 illustrates a typical experiment to demonstrate the development of immunity. Chickens infected with daily doses of 20,000 oocysts of *Eimeria acervulina* showed an increasing number of oocysts in faecal counts up to the 8th day when the number dropped rapidly. A 'challenge' of a million oocysts on the 21st day resulted in no increase in the number of oocysts, clearly demonstrating the development of immunity. The nature of the antigenic material is not known.

Mammalian coccidia. The two most important genera occurring in mammals are:

*Isospora*—oocyst with two sporocysts each with four sporozoites.

*Eimeria*—oocyst with four sporocysts each with two sporozoites.

*E. stiedae* from the rabbit is likely to be the most readily obtainable laboratory material, but other species are not uncommon. Many species of *Eimeria* are pathogenic (especially those whose schizonts release large numbers of merozoites) causing great economic

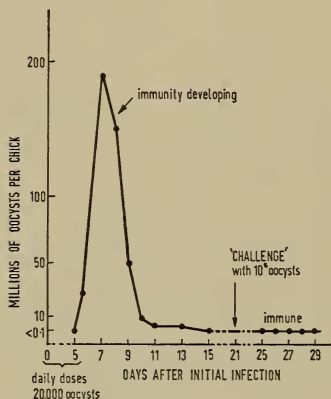


FIG. 24. Development of immunity by chickens against *Eimeria acervulina*. Initial parasitemia was produced by daily doses of 20,000 oocysts and the immune reaction became apparent on the 8th day. A 'challenge' of  $10^6$  oocysts on the 21st day demonstrated the effectiveness of the immunity (after Cuckler and Malanga, 1956).

loss to raisers of poultry, rabbits and cattle. In *E. tenella*, for example, the cause of the deadly coccidiosis in chicks, each schizont produces about 900 merozoites. In *E. bovis* of cattle, enormous schizonts, 250–400  $\mu$  in diameter and visible to the naked eye, are produced.

Two species occur in man, *Isospora hominis* and *I. belli*. These produce only mild symptoms, which last but a few weeks, since an immunity is probably rapidly developed. Only a few hundred cases have been reported.

*Coccidia with alternation of generations.* The family *Aggregatidae* contains species characterised by an alternation of generations between hosts which are marine annelids, molluscs or crustaceans. The best known life cycle is that of *Aggregata eberthi* with schizogony in the shore crab *Portunus depurator* and sporogony in *Sepia officinalis*. When ingested by the crab, each spore releases three sporozoites which grow and produce merozoites by schizogony within the peri-intestinal connective-tissue cells. When a crab is eaten by a cuttlefish, the merozoites penetrate the gut wall and develop into micro- and macrogametes; anisogamy occurs as in *Eimeria* and zygotes result. Each zygote nucleus is diploid, but by repeated divisions it produces many sporoblasts which become transformed into spores, each with three haploid sporozoites.

*Physiology.* The physiology of the Gregarinida and the Coccidia has been little studied. It is known that *Eimeria* oocysts require oxygen for their normal development.

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## CHAPTER VII

### SPOROZOA:

### HAEMOSPORIDIA; MALARIA

#### 7.1 General Considerations

As an environment, the vertebrate red blood cell has certain advantages to offer. It is thin-walled and in constant motion, with the result that absorption of food materials and elimination of waste products of metabolism are relatively easily accomplished. In addition, it contains rich supplies of protein and oxygen. These very features which make red blood cells efficient metabolising units thus similarly equip them to serve as admirable habitats for parasites.

The Haemosporidia are the only protozoan parasites capable of invading the red blood corpuscles of vertebrates; many species, if not all, have multiplicative phases in the reticulo-endothelial system. In spite of the importance of some of the species (i.e. those causing human malaria), and the vast amount of research that has been done upon them, many phases of the life cycles are either imperfectly known or entirely unknown.

All known forms have obligate heteroxenous cycles involving alternate vertebrate and arthropod hosts; formation and syngamy of gametes takes place in the latter. It is usually stated that the part of the cycle within the vertebrate host is *asexual*, and the portion within the invertebrate host is *sexual*, and on this basis the vertebrate and invertebrate hosts are often referred to as intermediate and definitive hosts respectively. These conclusions are probably not justified, for much depends on the position of meiosis in the life cycle and this is very imperfectly known for the group (but see p. 90). Since these terms are questionable, the terms *vertebrate* and *invertebrate* host are more appropriate in the present status of our knowledge.

All Haemosporidia undergo the same general type of developmental cycle which involves:

(a) initial or continual schizogony in the vertebrate host with initiation of gametogeny.

(b) formation of gametes in the arthropod host and subsequent fertilisation and formation of a zygote.



(c) formation of sporozoites from the zygote by repeated nuclear divisions followed by cytoplasmic divisions.

Since the transfer between vertebrate and invertebrate host is made by withdrawal or injection of parasites during the bloodsucking act, there are no resistant stages exposed to the hazards of the outside world, thus the production of protective spore cases, such as occur in coccideans, are unnecessary for survival.

The status of knowledge of the different phases of the three best-known genera is shown in Table 14 and it is evident that large gaps in our knowledge exist. The invertebrate hosts have been extensively studied in the case of the human malarial organisms, but are unknown for the chiropteran and reptilian *Plasmodium*, as well as for many species of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* of birds. The relative ease with which the phases in the blood stream may be studied has led to a disproportionate amount of attention being paid to their study compared with the other phases in the fixed tissues of the vertebrate host. In the case of the genus *Plasmodium*, these tissue stages remained undiscovered for nearly fifty years after the discovery of the erythrocytic parasites, yet their existence might reasonably have been predicted on theoretical grounds on the basis of the general similarity of this genus to the genera *Leucocytozoon* and *Haemoproteus*, in which such tissue phases were long known to occur.

## 7.2 Classification

### Family 1. *Plasmodiidae*

Genus *Plasmodium*: Parasites of mammals, birds and lizards. Exo-erythrocytic schizogony, sexual reproduction in blood-sucking insects.

### Family 2. *Haemoproteidae*

Schizogony in endothelial cells of vertebrates; no erythrocytic schizogony, gametocytes in erythrocytes or lymphocytes.

Genus *Leucocytozoon*: Parasites of birds; schizogony in visceral and endothelial cells of vertebrates; gametocytes in white blood cells.

Genus *Haemoproteus*: Parasites of birds and reptiles; gametocytes in erythrocytes, with pigment granules, halter-shaped when fully formed.

### Family 3. *Babesiidae*. Minute non-pigmented parasites of the erythrocytes of mammals.

Genus *Babesia*: In erythrocytes of cattle; pear-shaped, arranged in couples; sexual reproduction in female ticks.

TABLE 14  
STATUS OF KNOWLEDGE CONCERNING THE DIFFERENT PHASES OF VARIOUS MALARIA AND  
MALARIA-LIKE PARASITES

the use of the terms 'known' and 'unknown' often needs qualifications not capable of being shown graphically		(based on Huff, 1949)					
Organism	Host	Gametogony	Sporogony	Pre-erythrocytic	Schizogony	Erythrocytic	Phanerozoic
<i>Plasmodium vivax</i>	.	++	++	++	++	++	++
<i>Plasmodium malariae</i>	.	++	++	?	?	++	++
<i>Plasmodium falciparum</i>	.	++	++	++	++	++	does not occur
<i>Plasmodium ovale</i>	.	++	++	++	++	++	?
<i>Plasmodium berghei</i>	.	++	++	++	++	++	++
<i>Plasmodium gallinaceum</i>	.	++	++	++	++	++	++
<i>Plasmodium mexicanum</i>	.	++	?	?	?	++	++
<i>Leucocytozoon</i>	.	+	++	?	?	does not occur	+
Avian <i>Haemoproteus</i>	.	+	+	?	?	does not occur	+
Reptilian <i>Haemoproteus</i>	.	+	?	?	?	?	?

Key: ++ = known; ? = unknown; +- = imperfectly known; + = known only in certain species.

\* The terminology used in the schizogony of *Plasmodium* is not appropriate to *Haemoproteus* and *Leucocytozoon*.

### *Of Uncertain Systematic Position*

Genus *Toxoplasma*: Minute intracellular parasites in leucocytes and endothelial cells of birds, mammals and reptiles.

Genus *Theileria*: Minute intracellular parasites in erythrocytes and endothelial cells of mammals.

### 7.3 Genus *Plasmodium*—the malarial organism

Parasites of the genus *Plasmodium* are responsible for the disease 'malaria' in both animals and man. A list of some species is given in Table 15. Although the species attacking man have been most extensively studied, considerable use has been made of species in laboratory animals, especially *P. knowlesi* in monkeys, *P. relictum*, *P. cathemerium* and *P. gallinaceum* in birds, and recently *P. berghei* in rodents. Species also occur in amphibians, reptiles and other mammals such as squirrels and bats, but these have not been much studied. A number of comprehensive texts dealing with theoretical and practical aspects of malaria are available (Boyd, 1949; Russell, 1952; Russell *et al.*, 1946).

#### 7.31 History

Human malaria has been recognised since the earliest period of man's recorded history, and the occurrence of mosquitoes trapped in amber suggests its prevalence in pre-historic times. A variety of names was used to describe the disease—the shakes; march, Roman, jungle, intermittent fever; ague; chills. It was early thought (with good reason!) that there was an etiological relationship between swamps and these fevers. Italians referred to the bad air in fever-producing areas as *mala aria*, written *mal' aria*, and some time in the middle ages, the apostrophe was dropped, producing the term *malaria* as we now know it.

The work of Laveran, Manson, Ross and others showed the occurrence of the developmental cycle in the blood corpuscles and the transmission through mosquitoes, and by the early part of the present century it was generally believed that the broad outlines of the life cycle were fully known. When sporozoites were injected by a mosquito bite, they were thought to enter red cells directly and undergo schizogony—the actual penetration of a corpuscle being described in detail by Schaudinn. This simple account of the schizogonic cycle was accepted for nearly thirty years and published and republished in text-books of medicine and zoology. This account could not explain two facts (a) that there was always a time lag of six to nine days after a mosquito bite, during which time the organisms could not be detected in the blood, and (b) that in certain types of malaria, relapses were more frequent than in others—as if there existed a hidden reservoir of infection somewhere in the body.

What Schaudinn actually saw must remain a mystery, for between 1937–48, first in birds, then in monkeys, and later in man, it was found that the sporozoites, on entering the blood, went not directly to the red blood cells, as formerly thought, but within half an hour were carried to the reticulo-endothelial system (usually the liver) where they underwent two schizogony cycles. After the second schizogony, the majority of merozoites re-entered the blood stream and began the classical erythrocytic cycle, but some re-entered liver cells. The discovery of these exoerythrocytic (EE) tissue forms provided a satisfactory explanation for the early disappearance of the sporozoites, the inability of freshly infected blood to be infective to another individual and the occurrence of relapses; it also brought to life cycle of *Plasmodium* into line with that of *Leucocytozoon* and *Haemoproteus*.

TABLE  
SPECIES OF MALARIA IN  
WITH SOME OF THEIR

Species Avian <sup>1</sup>	Host	Carrier	
		Natural	Laboratory
<i>P. gallinaceum</i> <sup>2</sup>	. . . . . jungle pheasant	. . . . . domestic fowl	? <i>Aedes aegypti</i>
<i>P. cathemerium</i> <sup>2</sup>	. . . . . English sparrow	. . . . . canaries	? <i>Culex pipiens</i>
<i>P. relictum</i> <sup>2</sup>	. . . . . English sparrow	. . . . . canaries, pigeons	? <i>Culex pipiens</i>
<i>P. lophurae</i> <sup>2</sup>	. . . . . fire-back pheasant	. . . . . chickens, ducks	? ?
<i>P. rouxi</i>	. . . . . English sparrow	. . . . . canaries	? <i>Culex pipiens</i>
<i>P. vaughani</i>	. . . . . American robin	. . . . . canaries	? ?
<i>P. elongatum</i>	. . . . . English sparrow	. . . . . canaries	? ?
<i>P. fallax</i>	. . . . . tufted guinea fowl	. . . . . turkeys, pigeons	? <i>Aedes albopictus</i>
<i>Monkey</i>			
<i>P. inui</i>	. . . . . monkeys of genus <i>Macacus</i>	. . . . . <i>Macacus</i>	? <i>Anopheles maculipennis</i>
<i>P. cynomolgi</i> <sup>2</sup>	. . . . . ditto	. . . . . other lower monkeys easily infected	<i>Anopheles</i> spp. <i>A. spp.</i>
<i>P. knowlesi</i> <sup>2</sup>	. . . . . ( <i>Silenis irus</i> ) monkey	. . . . . ditto—also man	? ?
<i>P. kochi</i>	. . . . . monkeys of genus <i>Cercopithecus</i>	. . . . . monkeys of genus <i>Cercopithecus</i>	? ?
<i>Rodent</i>			
<i>P. berghei</i> <sup>2</sup>	. . . . . ( <i>Thamnomys</i> sp.) tree rat	. . . . . rats and mice	<i>A. dureni</i> see text
<i>Reptilian</i> <sup>3</sup>			
<i>P. rhadinurum</i>	. . . . . ( <i>Iguana iguana</i> ) lizard	. . . . . <i>Iguana</i> sp.	? ?
<i>P. mexicanum</i>	. . . . . ( <i>Sceloporus ferrariperezi</i> ) lizard	. . . . . ?	? ?
<i>P. pitmani</i>	. . . . . ( <i>Mabuia</i> spp.) skink	. . . . . ?	? ?
<i>Amphibian</i>			
<i>P. bufonis</i>	. . . . . ( <i>Bufo americanus</i> ) toad	. . . . . <i>Bufo americanus</i>	? ?

<sup>1</sup> Information given relates to infections produced by blood inoculations. With many species little information is available as to the character of the mosquito-induced infection.

<sup>2</sup> Most widely used for experimental work.

15

ANIMALS OTHER THAN MAN,  
CHARACTERISTICS

## Schizogony

cycle (hrs.)	Merozoite No.	Gametocytes	Comments	Locality
36	8-30	round or irregular	highly pathogenic	Ceylon
24	6-24	round or oval	periodicity v. marked	U.S. (Maryland)
24-26	8-32 <sup>4</sup>	round or oval	mildly pathogenic	Italy
24	8-18	elongate	highly pathogenic to ducks	Borneo (?)
24	4	elongate	parasitic level v. high; parasites easily found	Algeria
26	4-8	elongate	v. small; low parasitemia	U.S. (Michigan)
24	8-12	elongate	highly pathogenic to canaries	U.S. (Baltimore)
36?	?	halteridium-shaped	no periodicity	Sudan
72	12-16	about size of host cell	mildly pathogenic	East Indies
48	20	like <i>P. vivax</i>	mildly pathogenic	Malaya; Java
24	8-16	round	highly pathogenic except in natural host	Malaya
48?	? <sup>5</sup>	round	mildly pathogenic	E. Africa
22-24	8-16?	?	pathogenicity varies	The Congo
?	4	round, large	resembles <i>P. vaughani</i>	Mexico
?	19?	oval or elliptical	resembles <i>P. elongatum</i>	Mexico
?	10	bean-shaped	resembles <i>P. relictum</i>	Uganda
?	8	?	v. imperfectly known	Canada

<sup>3</sup> About 13 species known.<sup>4</sup> Varies with strains.<sup>5</sup> Not seen in peripheral blood.

### 7.32 General Features of the Malarial Life Cycle

As far as it is known, all plasmodia of animals, including man, spend a part of their life in a vertebrate and part in a mosquito host. It has been suggested that certain species of plasmodia of reptiles and bats may have arthropod hosts other than mosquitoes but this has not been confirmed. An excellent general review of the life cycle is given by Huff (1949).

There are four phases of development in the life cycle of most species of plasmodia; all phases are not known for every species.

(a) *Pre-erythrocytic* development from sporozoites injected into the host from a mosquito.

(b) *Erythrocytic schizogony* in the red cells.

(c) *Phanerozoic schizogony* in the reticulo-endothelial system.

(d) *The sexual cycle*, beginning with the growth of gametocytes in the vertebrate host and continuing with the sexual process followed by sporogony in the mosquito.

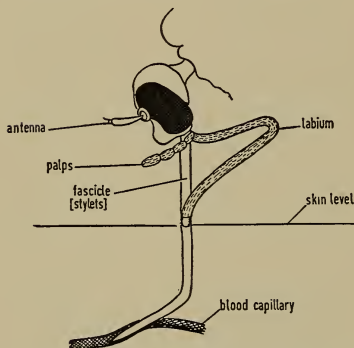


FIG. 25. Procedure adopted by *Aedes aegypti* on biting a frog's foot. Note how the fascicle is introduced directly into a capillary (from Boyd, 1949, after Gordon and Lumsden, 1939).

#### *Asexual Development in the Vertebrate Host*

*Pre-erythrocytic schizogony.* A generalised diagram of the life cycle is given in Fig. 26. When an infected mosquito bites man, numerous sporozoites from several hundreds or more may be injected. Since a mosquito usually feels about with its proboscis until it strikes a small capillary (Fig. 25) the sporozoites are probably introduced directly into the blood stream. A sporozoite is a minute slender fusiform organism with one or more chromatin masses near its centre causing a marked bulge. As discussed earlier, unreliable observations (by Schaudinn) had led to the belief that the sporozoites penetrated

directly into the red blood cells to initiate the erythrocytic cycle. It is now known that in at least five avian, one rodent, one reptilian, several monkey, and three human malarias, there are intervening stages—referred to in general as *exo-erythrocytic stages* (EE), a term introduced by James and Tate (1938)—during which the parasites live in fixed tissue cells and not in erythrocytes or reticulocytes. The development of these



# ANOPHELES MOSQUITO

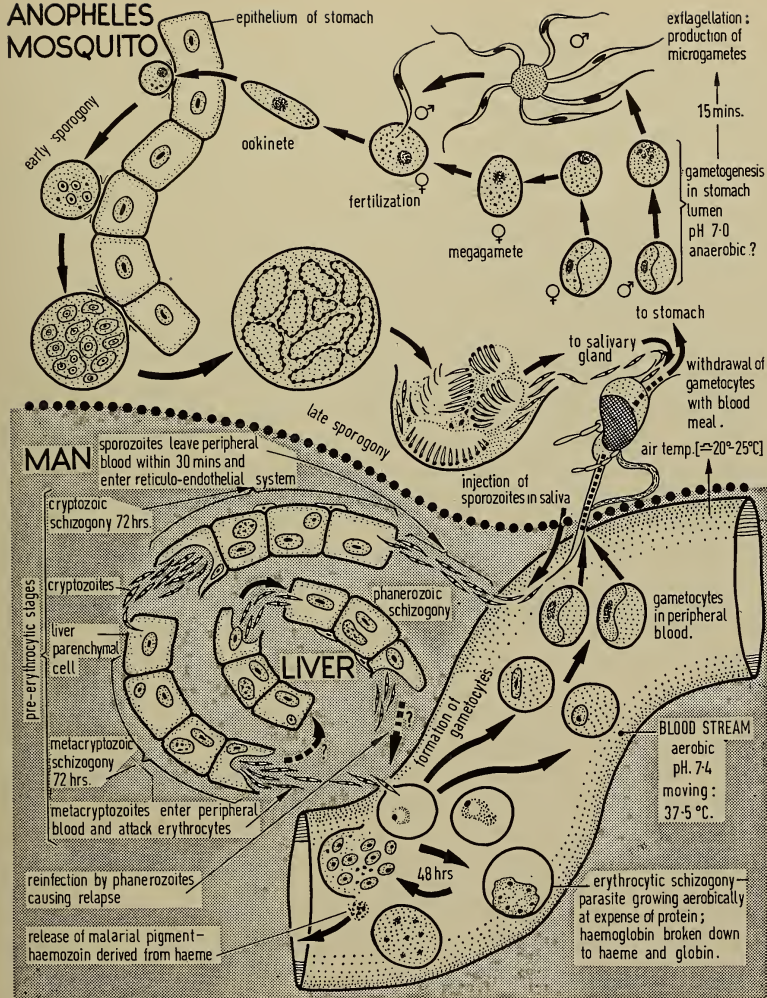


FIG. 26. Life cycle of the malarial organism based mainly on *Plasmodium falciparum* and *P. vivax*. (Phanerozoic schizogony does not occur in *P. falciparum*.) Original.

stages in all the *Plasmodium* species of man is not completely known, and has been fully worked out only in birds. Although there are species differences, the general pattern appears to be similar in all species studied. For the early references dealing with this phase, see Huff *et al.* (1948).

Injected sporozoites circulate in the blood for about half an hour after inoculation and then disappear; after this period the blood is not infective until after the end of the incubation period. On leaving the blood stream, the sporozoites enter fixed tissue cells (usually those of the liver) and undergo two schizogonic cycles before reinvading the blood stream. The time taken for the erythrocytic stages to appear varies with the species, being eight days for *P. falciparum*, six days for *P. vivax*, and seventy-five hours for the avian *P. gallinaceum*. The site of the exoerythrocytic schizogony also varies. *P. falciparum*, *P. vivax* and *P. ovale*—the three species infecting man in which the tissue stages have been fully confirmed—undergo exoerythrocytic stages in the liver, as do several species of bird malaria and the monkey form *P. cynomolgi*; the rodent form *P. berghei*, however, enters the reticulo-endothelial cells of the brain, lungs, heart and kidney. After several schizogony cycles are completed, the released organisms attack the erythrocytes and erythrocytic schizogony takes place. In some species, the exoerythrocytic forms appear to multiply almost indefinitely (particularly in avian species), but in others all the parasites are expelled into the blood stream within a few weeks. In *P. vivax* and *P. malariae* infections, for example, fixed tissue forms appear to be able to persist for a period and to invade the blood stream, causing relapses, as soon as a falling off in immunity or drug level permits them. This is an important point, for in *falciparum* this does not occur, and complete eradication of the blood forms by drug therapy ends the disease and relapses cannot occur. Those species which (up to the present) have been shown to possess EE stages fall into two natural groups, the *elongatum*-type and the *gallinaceum*-type named after avian species. In the *elongatum*-type, the EE stages appear as morphologically identical in a great variety of blood and blood-forming cells; in the *gallinaceum*-type, the EE stages possess a schizogonic series morphologically different from the erythrocytic series and which live predominantly in the reticulo-endothelial system and in true endothelium.

The discovery of the tissue forms, and the fact that they can undergo cycles of schizogonic multiplication before invading the blood stream or may revert to fixed tissue forms after undergoing erythrocytic schizogony, has led to the development of a complex nomenclature for the various types.

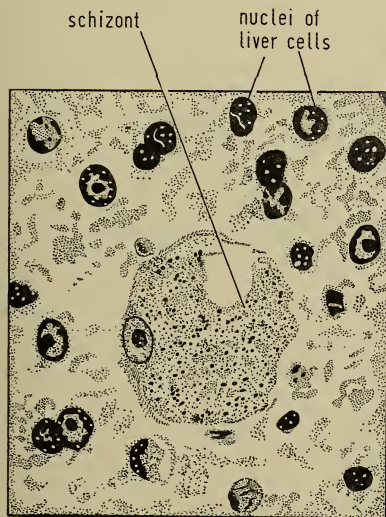
*Exoerythrocytic* (EE). A general term employed to describe stages exclusive of erythrocytic ones.

*Cryptozoites*. Exoerythrocytic stages which arise directly from sporozoites.

*Metacryptozoites*. Organisms of subsequent generations occurring before the appearance of parasitemia. Some of these attack red cells to initiate the erythrocytic schizogony.

*Pre-erythrocytic stages*. Cryptozoites and metacryptozoites collectively constitute the pre-erythrocytic stages.

*P. cynomolgi*



*P. vivax*

FIG. 27. Pre-erythrocytic schizonts of *Plasmodium cynomolgi* and *P. vivax* in parenchymal cells of rhesus monkey and man respectively; 7 days after mosquito inoculation (redrawn from Boyd, 1949).

*Phanerozoites*. EE forms appearing concurrently with subsequent erythrocytic stages. Thus the term *phanerozoic* would be applied to stages reinvading fixed tissue cells; it would also be used for EE stages in blood-induced infections in which the sporozoites do not take part.

The pre-erythrocytic schizonts of many species contain an enormous number of merozoites—about 40,000 in the case of *P. falciparum*, but much less, about 1,000, in *P. vivax* and *P. cynomolgi* (Fig. 27).

*Erythrocytic schizogony* (Fig. 26). On release from fixed tissue cells, the metacryptozoites attack red corpuscles in which they first appear as a minute speck of chromatin surrounded by scanty protoplasm. It is generally assumed that the erythrocytic plasmodium is intracellular but some observers consider it may be attached to the outside membrane of the erythrocyte, at least for a time. The uninucleate plasmodium gradually becomes ring-shaped and is known as a ring or trophozoite. It grows at the expense of the erythrocyte and assumes a form differing widely with the species but usually exhibiting active pseudopodia. Pigment granules (formula p. 92) appear early in the growth

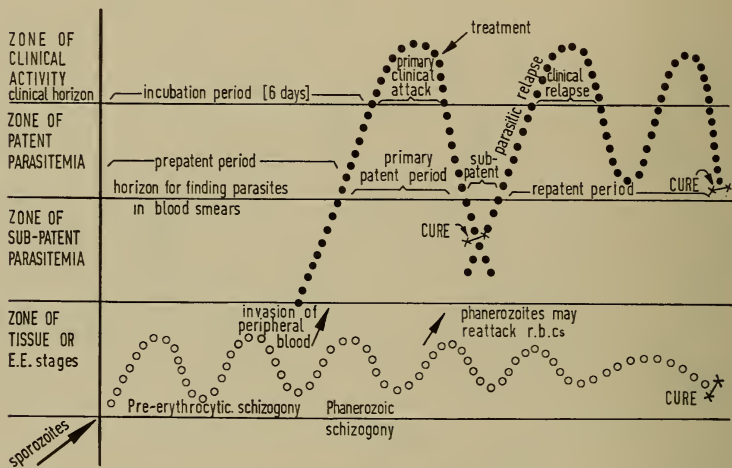


FIG. 28. Diagram illustrating some relationships between the malarial parasite and its host (modified from Russell, 1952).

phase. As the chromatin begins to divide, the parasite is known as a schizont. The dividing chromatin tends to take up peripheral positions, and a small portion of cytoplasm gathers around each. The mature schizont is often known as a *segmenter*. The infected erythrocyte ruptures and releases a number of merozoites which attack new corpuscles, thus repeating the cycle of erythrocytic schizogony. The infection about this time enters the phase of *patent parasitemia* (Fig. 28) with parasites detectable in blood smears.



In some species, merozoites show a distinct predilection for erythrocytes of a certain age. Many avian species attack almost exclusively the young erythrocytes. In the human plasmodia, the merozoites of *vivax* attack young immature corpuscles (reticulocytes), those of *malariae* attack the older ones, and those of *falciparum* indiscriminately enter any available. This partly (but see p. 97) explains the virulence of *falciparum* infections in which 10 per cent or more of the erythrocytes may be attacked, whereas *vivax* seldom occurs in even 1 per cent of the corpuscles, and *malariae* seldom in more than 0.2 per cent. Schizogony may continue for many months or even years; some cases of *P. malariae* have been recorded in which the infection seems to have persisted for 30–40 years.

All segmenters release their contained merozoites within a period of several hours together with a pigment and other waste products, and there is a sudden paroxysm of fever in the host characterised by a marked rise in temperature. This periodicity in reproduction is one of the most remarkable features of malarial organisms, human and avian species being particularly regular. The length of the cycle is usually some multiple of 24 hours. In *vivax*, *ovale* and *falciparum* it is 48 hours and in *malariae* it is 72 hours, but in some monkey species (e.g. *knowlesi*) and some avian species (e.g. *cathemerium*) it is 24 hours (Table 15).

The terms 'tertian' and 'quartan' as applied to human malarias are derived from the old Roman method of reckoning which counted the day on which something happened as the first day, the second day following being the third (tertian) and the third following day the fourth (quartan). In the early stages of *vivax* and *falciparum* infections, paroxysms of fever commonly occur daily (quotidian) an effect probably due to the fact that metacryptozoites are not all released into the blood stream at the same time.

*Formation of Gametocytes.* Some merozoites on entering red cells become sexual gametocytes, instead of asexual segmenters. Male gametocytes are termed *microgametocytes*, and female *macrogametocytes*. Little is known concerning the factors influencing gametocyte production but sexual forms may occur at any stage of an infection, and possibly may be formed from both exoerythrocytic and erythrocytic stages. It seems that the fate, sexual or asexual, of any given merozoite is probably determined at the time of its formation. The only factors known to affect gametocyte formation are the physiological conditions of the host, the type of host and the method of transmission (i.e. whether by syringe or mosquito passage). The type of host is particularly important and infections of bird malaria in heterologous hosts, such as rodents, may result in a parasitemia in which gametocytes are not produced. In some strains, however, a change in the numbers of gametocytes produced appears to be inherent in the strain itself. Thus *P. nucleophilum* (from canaries) when inoculated into ducks, failed to produce gametocytes but it also ceased to produce them when reintroduced into canaries.

Again, some drugs appear to inhibit gametocyte production and others encourage it. Although results such as these are pointers, it may generally be considered that the basic mechanisms behind gametocyte production are not understood. Bishop (1955) has reviewed the problem.

### *Asexual cycle in the mosquito*

If a mosquito of a susceptible species sucks up blood containing mature gametocytes, these develop into male and female gametes. This process, in male gametocytes termed *exflagellation*, is extremely rapid, and may be completed within 10–15 minutes.

It is readily seen under the microscope, if a drop of blood containing gametocytes, mixed with saline-citrate to prevent clotting, is placed on a slide. The microgametocyte extrudes six to eight long flagella-like microgametes, each containing nuclear material. These remain attached for a few minutes, lashing actively until liberated, when they swim away seeking female macrogametes.

The factors controlling exflagellation are unknown; pH, O<sub>2</sub> or CO<sub>2</sub> tension do not appear to be directly responsible (Bishop and McConnachie, 1956). Maturation of the female gametocyte is less striking, it extrudes Feulgen-positive maturation bodies before fertilisation. The production of these bodies is usually assumed to provide evidence of a pre-gametic reduction division. This view, cannot be unreservedly accepted as 'maturation' bodies have been described in other protozoa in which no reduction in the number of chromosomes takes place. Although the genetics of *Plasmodium* are not properly understood, there is recent evidence that meiosis occurs in the early oocyst (Bano, 1958).

A microgamete fertilises a macrogamete and the resulting zygote develops into a mobile elongated ookinete. This penetrates the gut wall of the mosquito between the cells and develops as an oocyst between the epithelium and the basement membrane. The oocyst matures in 10–20 days depending on temperature, species and perhaps individual mosquitoes, growing to a body 50–60  $\mu$  in size. The chromatin divides repeatedly until there are hundreds of tiny nuclear masses, and the cytoplasm follows suit resulting in the production of enormous numbers of thread-like sporozoites. When mature, an oocyst bursts and the released sporozoites make their way to the salivary glands where they become intracellular or extracellular organisms, or remain free in the ducts.

Extensive investigations have shown that although avian, rodent and simian species differ somewhat in certain details of the morphology and life cycle, these differences are not very great and the general picture given above can be accepted as the basic pattern of the life cycle.



### 7.33 Physiology

*General Metabolism.* This field has been reviewed by Moulder (1955) and McKee (1951). The majority of physiological and metabolic studies carried out on plasmodia have been concentrated on the erythrocytic stage which is the one most readily obtainable for study. The metabolic processes of the sporozoites and EE stages on the other hand have received little attention. Until comparatively recently, lack of suitable *in vitro* culture techniques for plasmodia have been a considerable stumbling block to investigations.

Biochemically, malarial parasites are complex bodies containing ribo- and deoxyribonucleic acids, lipids, polysaccharids and proteins. They have high metabolic rates—higher than their erythrocyte host. They also possess batteries of enzymes which, as far as is known, duplicate every vertebrate enzyme system tested for.

*Respiration.* Respiration is mainly aerobic, the organisms obtaining their main supply of oxygen from oxyhaemoglobin. Oxygen utilisation varies greatly both with species and size. The oxygen uptake of parasitised cells is anything from 25–100 times greater than that of unparasitised erythrocytes. For example, the oxygen consumption of normal monkey erythrocytes rises from 0.73 m.M. per hr./ $5 \times 10^{12}$  cells to 51.0 m.M. in parasitised cells, but varies with the oxygen tension in the atmosphere. Excess oxygen appears to be detrimental to respiration.

*Respiratory enzymes.* Respiration of *P. knowlesi* and *P. lophurae* is almost completely inhibited by 0.001 M. cyanide, and considerably (about 64 per cent) by carbon monoxide. The effect of light has given inconsistent and inconclusive results. Thus, as in the case of the other blood-inhabiting protozoans (e.g. trypanosomes, p. 67) the presence of iron-porphyrin respiratory enzymes is indicated. There is also some evidence for the presence of flavoprotein systems.

*Carbohydrate metabolism.* Although many carbohydrates (glucose, mannose and fructose) can be oxidised by the parasite, only glucose can satisfy the long-term requirements of growth and reproduction. The parasitised cell utilises enormous quantities of glucose, some 25–100 times that consumed by the normal cell. Glycerol can also act as a substrate for short periods and shows a higher oxygen utilisation, but it will not maintain the parasites in 24-hour cultures. In *Plasmodium gallinaceum*, it has been shown that glucose is quantitatively converted into lactic acid under anaerobic conditions and most of the enzymes of the classical Embden-Meyerhof sequence have been detected, indicating the functioning of a typical muscle type of phosphorolative glycolysis. In cultures, the metabolising of glucose leads to accumulation of lactic acid and unless this acid is

neutralised the respiratory activity of the parasites is impaired (see *in vitro* cultivation, p. 404).

The oxidative process in the carbohydrate metabolism undoubtedly involves the Krebs tricarboxylic-acid cycle which is primarily a series of decarboxylation and dehydrogenation processes. The malarial organism, however, is wasteful in its energy-producing mechanisms, since it glycolyses much more carbohydrate than it oxidises and lactic acid accumulates as pointed out above. Although the anaerobic glycolytic process is a relatively inefficient one, in this instance it probably supplies about 10 per cent of the total energy, the remaining 90 per cent or so coming from the enzymatic transfer to oxygen of the electrons liberated in reactions given above. If the evidence in favour of the iron-porphyrin and flavo-protein system of enzymes is accepted, presumably the hydrogen is passed first to DPN (diphosphopyridine nucleotide) then to FAD (flavine

adenine dinucleotide) and finally to molecular oxygen via the cytochromes.

**Protein metabolism.** The organisms contain enzyme systems which can split red-cell haemoglobin into a non-protein moiety and globin, about 76 per cent of the haemoglobin in a red cell being destroyed during the life of the parasite. The non-protein portion, which is haematin, is not utilised but is released as the characteristic malarial pigment ('haemozoin' =  $C_{33}H_{42}N_4O_4FeOH$ ) when the parasites rupture the ery-

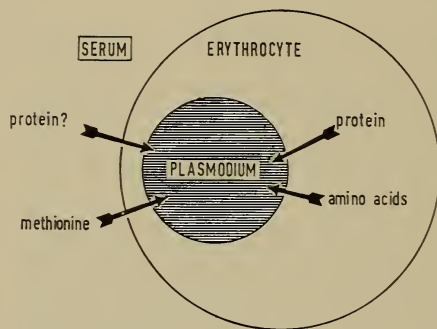


FIG. 29. Probable sources of parasite protein in *Plasmodium knowlesi* (modified from Moulder, 1955).

throcyte as they escape. Although plasmodia undoubtedly obtain almost all their amino acids from the breakdown of host-cell protein (Fig. 29), there is one important exception, at least in *P. knowlesi*. This is methionine, which is essential for plasmodial growth, and which is present in haemoglobin only to the extent of about 1 per cent whereas most proteins contain 3-4 per cent of this amino acid. The red-cell protein is thus deficient in methionine and the parasite is forced to draw on this substance from the surrounding plasma. If this is true for other species of plasmodia, it is clear that although these organisms are morphologically parasites of the host erythrocyte, they are physiologically parasites of other cells and the plasma. It has been shown that

even *in vivo* there is a need for extra plasma methionine since monkeys infected with *P. knowlesi* require methionine in their diet for normal growth of the parasite.

The bulk of the amino acids and peptides are utilised for synthesis and only very small amounts are utilised in oxidative processes.

*Lipid Metabolism.* Little knowledge of this aspect of plasmodia physiology is available. Erythrocytes infected with *P. knowlesi* show more than 400 per cent increase in the total lipids, a figure representing about 28.8 per cent of the dry weight of the isolated parasites. Some 25 per cent of the lipid is non-saponifiable material, mainly cholesterol. The fatty acid content of *P. knowlesi*-infected cells is about 4–5 times that of the normal cells, and the presence of an unsaturated monocarboxylic fatty acid with 18 carbon atoms and lytic powers has been reported. It is possible that the accumulation of this C-18 fatty acid is related to the rupture of the erythrocyte envelope and the release of the merozoites.

*Growth factors.* There is evidence from *in vivo* and *in vitro* studies that in addition to the amino acid methionine, ascorbic acid and *p*-aminobenzoic acid are important dietary factors. The lack of ascorbic acid in cultures has no appreciable effect on the multiplication of the parasite and this factor, at least, acts directly through the host animals. The need for *p*-aminobenzoic acid is not understood.

*In vitro Cultivation.* The problem of intra-cellular and extracellular cultivation of plasmodia is discussed on p. 404.

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## CHAPTER VIII

### SPOROZOA :

### MALARIA IN THE ANIMAL KINGDOM

#### 8.1 Mammalian Malaria

##### 8.11 Human Malaria

*Importance.* It is not always realised that malaria is the world's greatest killer, not giving place even to cancer or heart disease. It has been estimated that there are 250,000,000 cases of the disease annually throughout the world, of which some 3 million are fatal! And yet, ironically enough, it is one of the few diseases in which the life cycle of the causal parasite is completely known and for which methods of destroying or preventing each stage of the cycle are available. The limitations on the anti-malaria measures are mainly financial and physical, the enormous areas concerned calling for superhuman efforts to bring them under control, but in undeveloped areas, ignorance and superstition also present appalling difficulties. Yet, in areas where financial support has been available and intelligent use made of available methods, the incidence has dropped, in some instances from 2,000 cases per 1,000 per year to 50-100 per 1,000. In the U.S.A., malaria, which was once prevalent, has been virtually eliminated. In the decade 1935-45, mortality from malaria dropped by over 90 per cent, and in 1951 the National Malaria Society voluntarily dissolved itself because its goal, the elimination of malaria as an endemic disease, had ceased to exist. An excellent general account of human malaria is given by Russell (1952).

Four species of *Plasmodium* infect man naturally and result in four kinds of malarial fevers:

*P. vivax*: benign, simple or tertian malaria.

*P. falciparum*: aestivo-autumnal, malignant tertian, pernicious quotidian, subtertian or tropical malaria.

*P. malariae*: quartan ague, or quartan malaria.

*P. ovale*: ovale tertian malaria.

TABLE 16

## COMPARATIVE CHARACTERS OF PLASMODIA OF MAN. MORPHOLOGICAL DESCRIPTIONS BASED ON STAINED THIN FILMS

	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. ovale</i>
Incidence in world malarial infections . . .	50 per cent	43 per cent	7 per cent	rare
Erythrocytes attacked . . .	any indiscriminately	reticulocytes only	older erythrocytes only	?
Exoerythrocytic forms (hrs) . . .	do not persist	persist	persist	?
Schizogony cycle . . .	48	48	72	48
Rings . . .	$\frac{1}{3}$ – $\frac{1}{2}$ diam. r.b.c. Multiple infections common	$\frac{1}{3}$ diam. r.b.c. Often 2 rings or more per r.b.c.	$\frac{1}{3}$ diam. r.b.c. Double infections rare	as <i>malariae</i>
Late trophozoite . . .	Medium, compact; band forms frequent; vacuole inconspicuous; rare in peripheral blood	Large, very amoeboid; prominent vacuole	Small, not amoeboid; often band-shaped; vacuole inconspicuous	Small, not amoeboid; vacuole inconspicuous
Mature segmenter . . .	Smaller than r.b.c. Rare in peripheral blood	Larger than r.b.c.	Smaller than r.b.c.	Larger than <i>malariae</i>
Number of merozoites . . .	12–24, usually 8–12	8–16, usually 12–15	6–12, usually 8	6–12, usually 8
Microgametocytes* . . .	Crescents usually sausage-shaped	Spherical; compact	Similar to <i>vivax</i> but smaller and less numerous	as <i>malariae</i>
Macrogametocytes . . .	Crescents often longer and more slender	Spherical; compact	Similar to <i>vivax</i> but small and less numerous	as <i>malariae</i>
Alterations in r.b.c. . .	Normal size; appear 'brassy'. Maurer's dots or 'clefs' may be visible; rare in peripheral blood	Enlarged and pale; Schüffner's dots present	May appear smaller; fine dots (Ziemann's dots) occasionally seen	r.b.c. oval; Schüffner's dots prominent and appear early

\* Usually smaller and less numerous than macrogametocytes.

The pattern of the life cycles of these species follows the general outline already given (p. 84) but there are important physiological differences which are often reflected in the nature of the diseases produced. Some of the morphological characteristics are summarised in Table 16 and illustrated in Fig. 30.

Many of these characteristics are sufficiently definite to be used for criteria for identification in blood films, but species differentiation may not be possible in the

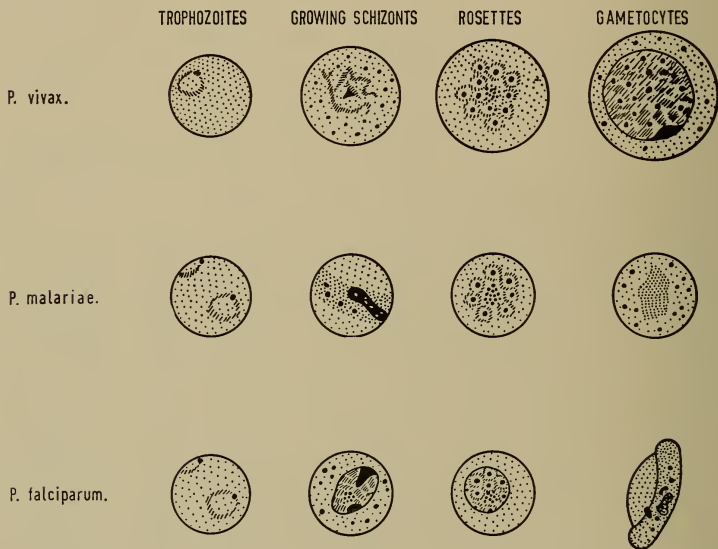


FIG. 30. Comparison of the various stages of the three common species of *Plasmodium* infecting man (after various authors).

earliest stages. In *vivax* and *ovale* infections, erythrocytes become enlarged and paler in colour when the parasites have grown beyond the ring stage. In stained preparations, infected corpuscles show characteristic dots called Schüffner's dots (*vivax* and *ovale*), Ziemann's dots (*malariae*) or Maurer's dots (*falciparum*). The pigment granules vary in size and shape but their appearance and colour depend on a number of factors such as intensity of light, type of filter used, etc.



*Characteristics of species*

*P. falciparum*. The most important malarial parasite, the disease it produces running an acute course and often terminating fatally. It is responsible for some 50 per cent of all the malarial cases throughout the world. Its distribution is restricted to warm and tropical countries.

The special feature of its life cycle may be summarised as follows:

(a) It attacks erythrocytes of all ages indiscriminately so that a high density of parasites may be rapidly reached. In extreme cases up to 48 per cent of the red cells may be parasitised.

(b) Multiple infections (polyparasitism) resulting in several ring forms in a corpuscle are not uncommon.

(c) The later stages in the asexual cycle, that is, the growth to schizonts, do not occur in the peripheral blood as in other forms of malaria, except in severe cases, so that only rings and crescents are found in blood films. After twenty-four hours, the ring forms and older trophozoites show a tendency to clump together and adhere to the visceral capillary walls and become caught up in the vessels of the heart, intestine, brain or bone marrow in which the later asexual stages are completed. This behaviour, together with the fact that the subtertian malaria is more toxic, are the principle reasons why this type is so dangerous.

(d) Sporulation is not so well synchronised as in other species so that fever paroxysms may be longer drawn out.

(e) EE forms do not persist in the tissues and hence relapses do not occur.

*P. vivax*. Causes the benign tertian form of malaria which is responsible for about 43 per cent of all cases in the world and has the widest geographical distribution. Several points in its life cycle may be noted:

(a) The degree of infection is low, for only the young immature corpuscles (reticulocytes) are attacked; about 2 per cent of erythrocytes are parasitised.

(b) The periodicity of the asexual cycle is closely synchronised.

(c) The EE stages persist, so that relapses can occur.

*P. malariae*. A relatively rare parasite producing quartan malaria which is responsible for about 7 per cent of the malaria in the world. Particular points of interest are:

(a) Infected erythrocytes are not larger than uninfected ones and sometimes even smaller.

(b) Mature erythrocytes are attacked and rarely reticulocytes, so that the density of parasites is very low; about 0.2 per cent erythrocytes parasitised.

(c) EE stages persist and relapses can occur.

(d) It is often difficult to distinguish between a large trophozoite and an immature gametocyte.

*P. ovale*. This is a species rarely encountered. The type of fever it produces (ovale tertian) is milder than the benign tertian of *vivax*. Special points of interest are:

(a) It morphologically resembles *P. malariae* in most of its stages.

(b) The changes produced in the erythrocytes in general are similar to those produced by *P. vivax*, but Schüffner's dots appear considerably earlier (in the ring stage) and are coarser and more numerous.

(c) In the oocyst the pigment granules are (usually) characteristically arranged in two rows crossing each other at right angles.

(d) Occurrence of relapses has been little studied in this species.

*Vectors of human malaria*. As far as is known, the plasmodia of man will develop only in mosquitoes of the genus *Anopheles* and not in any other arthropod. Almost any species can be infected with plasmodia in the laboratory, but many species are poor vectors and not natural ones. For a mosquito to be an efficient vector, it must present certain characteristics: (a) it must be susceptible to infection and present physico-chemical and nutritional characteristics suitable for the development of plasmodia, (b) it must bite man in preference to animals, (c) it must not be shy of human habitation, (d) its span of life must be sufficiently long to permit sexual development of the plasmodium.

It is not intended to deal here with the morphological or physiological features of mosquitoes, or to provide taxonomic details. These are fully treated in reference works such as those of Russell, West and Manwell (1946), Russell (1952) and Boyd (1949).

*Immunity*. Immunity, when it develops in man is not only species-specific but also strain-specific, for each species of *Plasmodium* is a complex of immunologically different strains. Although considerable work has been carried out on the question of acquired immunity to malaria, the mechanism is complicated and not properly understood. The question is further discussed on p. 382.

## 8.12 Monkey Malaria

Some of the commoner species of malaria attacking monkeys are listed in Table 15. *Macacus (Silenus) rhesus* has been most extensively used as a host, young specimens being found the most suitable. The relationship between the plasmodia of man and monkeys is not properly understood. Most monkey species will not grow in man, but *P. knowlesi* is transmissible to man by blood inoculation and produces a mild and easily controllable malaria.

The cycle of these species and the pathology of the disease in monkeys differ little from their human counterparts. *P. knowlesi* behaves like human *falciparum* malaria, the infected erythrocytes clumping in the visceral blood with similar effects on the host. *P. cynomolgi* behaves very like *P. vivax*, and this species played an important part in the discovery of the EE forms. The pattern of immunity in monkeys appears to follow closely that of humans (p. 382).

### 8.13 Rodent Malaria—*P. berghei*

*Discovery.* In 1943, a Belgian antimalarial team began working on the mosquitoes of certain forest districts in the Belgian Congo. The mosquito *Anopheles durenti* which has a localised distribution (on shady trees by the Kisanga River) was found to be frequently gorged with non-human blood, and to show a high sporozoite index. This engorged blood when tested gave positive reactions to anti-rat serum, so the blood of rodents occurring in the district was examined for parasites. This led to the discovery of a new species of malaria, *P. berghei*, in the blood of the tree rat *Thamnomys surdaster surdaster* (Vincke and Lips, 1948). This was a finding of immense significance, for it has been found possible to transmit this species of malaria to a number of laboratory rodents with the result that laboratory research on malaria has been greatly facilitated.

*Vectors.* The natural host is *Anopheles durenti*, a species of mosquito peculiarly difficult to maintain in the laboratory. Many attempts have been made to infect other more easily kept species. No development occurs in the common laboratory mosquitoes *Culex pipiens* or *Aedes aegypti*, but some positive results have been obtained with *Anopheles maculipennis* var. *atroparvus*, *A. stephensi*, and *A. quadrimaculatus*. Laboratory transmission can, of course, be readily performed by intraperitoneal or intravenous injection, but the parasitemia produced by blood inoculation and sporozoite infection may show marked differences.

*Laboratory Hosts.* The following have been infected to a greater or lesser extent: rats, mice, golden hamsters, field voles and cotton rats. Hamsters, albino mice and albino rats are probably the most readily infected laboratory animals and may show infections up to 50 per cent, 90 per cent and 60 per cent erythrocytes parasitised respectively. Guinea pigs have a high natural resistance as have rabbits and in both these organisms, only a slight parasitemia develops. In all the above hosts immunity is fairly readily developed but varies with the species. There is evidence that immunity developed by female rats can be transmitted to their young via the milk.

*Morphology and Life Cycle.* The life cycle follows the typical pattern (Fig. 26). Both the morphology and life cycle are dealt with in detail by Sergeant and Poncet (1956) and Mercado and Coatney (1951). The literature has also been reviewed by Thurston (1953). The following points of interest may be noticed in mice infections:

- (a) The pre-erythrocytic cycle is probably 3 days. In blood-induced infections the

prepatent period is 3–6 days; the patent period 4–19 days; the peak period of parasitemia occurs at 3–5 days. Death is 100 per cent.

(b) The trophozoites have a definite predilection for reticulocytes up to 100 per cent of which may be infected, whereas the percentage of mature erythrocytes infected may be only about 10 per cent. In contrast, gametocytes develop in mature erythrocytes exclusively.

(c) Polyparasitism is not uncommon, the cytoplasm of the parasites merging into a confluent mass.

(d) Exflagellation is rapid, requiring only 10–30 minutes.

(e) The schizogony cycle is approximately 22–24 hours, and the number of merozoites 8–16.

(f) Occurrence of exoerythrocytic stages has not been fully confirmed, but may be like the avian type and confined to the reticulo-endothelial system.

(g) The gametocytes are prone to disappear in serial passage in mice but not in rats.

*Pathogenicity.* Varies considerably with the species of host from benign to acute pathogenicity.

## 8.2 Bird Malaria

Most of the important milestones in understanding the malarial life cycle were discovered using bird malaria as experimental material. Ross's discovery of the mosquito transmission was the most prominent contribution; but other substantial contributions include the introduction of various malarial drugs first tried out on birds, and more recently, the establishment of the extra-erythrocytic stages of the life cycle. A useful monograph of bird malaria is that of Hewitt (1940).

*Occurrence and Geographical Distribution.* Plasmodia in birds are widespread throughout the world, except possibly in the far North; the incidence varies within the range 1–19 per cent with a mean of 5·8 per cent (estimated on some 7,000 birds). The climatic barriers which limit the distribution of human malaria do not exist in bird malaria, and *the incidence of infection is as high in temperate regions as it is in the tropics.*

Passerine birds are more frequently infected than any other group, sparrows show the highest incidence of infection. Domestic birds, e.g. pigeons, geese, ducks, turkeys, chickens and doves, are rarely infected in nature.

*Experimental Hosts.* One of the difficulties that beset early workers was the fact that they could never be sure that the laboratory hosts which they used, mainly sparrows, chaffinches, pigeons, linnets, larks, were free from parasites. The discovery of the canary as a suitable laboratory host for most species of avian malaria soon led to its adoption as the standard experimental host. Canaries (especially females) have the advantage of

being cheap, easily maintained and virtually never infected naturally with malarial parasites. More recently, however, it has been replaced by the chick (see below), supplies of which may be usually cheaply obtained.

*Species.* There are about 40 species of avian malaria known, of which only a small number have been used extensively in laboratory studies. *Plasmodium relictum* and *P. cathemerium* (Fig. 31) have been most widely used for experimental work and they also occur

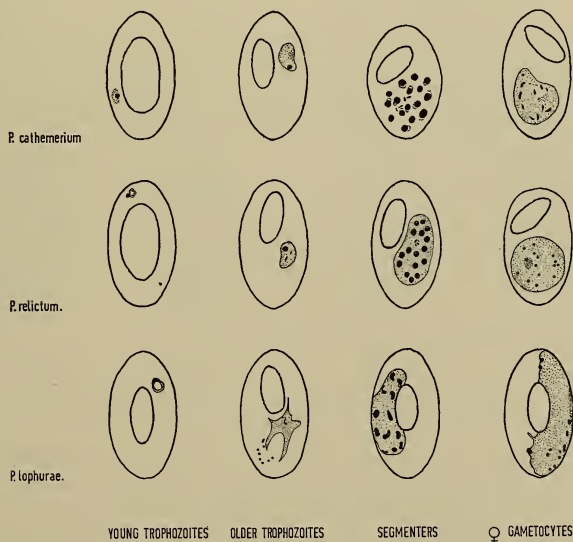


FIG. 31. Comparison of the various stages of three species of avian plasmodia (after Hewitt, 1940).

more commonly than any other species in nature. They are particularly suitable in being readily infective to canaries and transmissible by *Culex pipiens*, one of the few species of mosquitoes easily maintained in the laboratory. The small size of canaries does not permit the withdrawal of more than about 600–800 mg of blood, and since the total amount of blood only amounts to about 1,000 mg the donor always dies. Where large amounts of parasitised blood are required, more suitable laboratory species are *P. gallinaceum* or *P. lophurae* (Fig. 32). The former species was first imported into

France by Brumpt in 1936, in domestic fowl and is readily maintained in the laboratory in chicks.

When a malarial parasite is isolated from a wild bird and subinoculated into a laboratory host and carried through this host for several generations, it is usual to designate it a separate strain. There are, for example, at least twelve strains of *P. relictum* and some seven of *P. cathemerium*. One of the latter strains, for example, first described by Huff, is a 'gametocyteless strain', lacking sexual stages in its cycle, and showing some morphological differences from the typical *P. cathemerium*, as well as exhibiting a marked lack of synchronism in its asexual cycle.

### Life Cycle

*General account.* The life cycle of avian malarial parasites more or less follows the typical pattern already described (p. 84) with, of course, specific differences. The periodicity (Table 15) is never more than 48 hours, and is usually 24 hours, but strain as well as

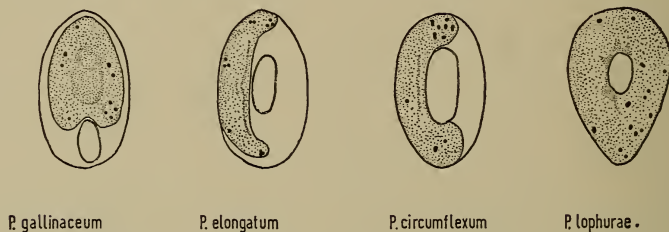


FIG. 32. Microgametocytes of four species of avian plasmodia (after Hewitt, 1940).

specific differences occur. In the asexual cycle, the type of red cells parasitised varies with the species. Details are lacking for some of the rarer species, but in the majority of common species immature cells (reticulocytes)—characterised by possessing large nuclei (Figs. 31, 32)—are more frequently parasitised. An exception is *P. elongatum* which is capable of living in all blood and blood-forming cells in the canary, including granular leucocytes, and segmenters occur more frequently in the bone marrow than in any other part of the body. A red cell normally matures in 24 hours, so that in the case of a species of avian *Plasmodium* with 24 hours periodicity, both the red cell and its contained parasite reach maturity at approximately the same time. The reason why young red cells are, in general, more susceptible to attack is not known. In species other than *P. elongatum*, parasites are commonest in the peripheral blood but considerable variation occurs and many visceral organs may contain concentrations of parasites at one time or another.

Morphologically, the erythrocytic stages of avian species differ in such features



as the shape of the young trophozoites and position within the cell, the number of merozoites in mature segmenters, the number and appearance of the pigment granules, and the shape of the gametocytes. Many of these features serve as useful criteria for the differentiation of species, especially the shape of the gametocytes, which appears to be a constant and dependable character. All the described species fall into two groups (a) those with round or oval gametocytes, and (b) those with elongate gametocytes. Most of the best known experimental species belong to the former group (Figs. 31, 32); *P. elongatum*, *P. vaughani* and *P. lophurae* are examples of the latter group.

The prepatent period in avian malaria varies with the species, the method employed in inoculation (i.e. by mosquito bite, sporozoite injection or blood transfer) and particularly the size of the inoculum. With heavy inocula ( $2 \times 10^7$  organisms) the prepatent period may be as short as one day, with light injections ( $1 \times 10^3$ ) as long as nine days. The length of the patent period similarly varies with the nature of the infection, whether benign or acute, and covers the range 5–23 days. The subpatent periods in bird malarias may be as long as eight months. During this period, parasites are present in such small numbers that they cannot be demonstrated by the usual routine methods in the peripheral blood, and their presence can only be confirmed by subinoculation into other unparasitised animals.

As pointed out earlier (p. 86), the division of malaria species into two natural groups has been based on a study of the EE stages in bird species. In the *elongatum*-type the EE stages appear as morphologically identical stages in a great variety of blood and blood-forming cells. In the *gallinaceum*-type, the EE stages possess a schizogonic series morphologically different from the erythrocytic series and which live predominantly in the reticulo-endothelial system and in true endothelium.

*Exoerythrocytic schizogony.* The extent to which exoerythrocytic stages occur in experimental animals depends on the method of transmission. In the case of *P. gallinaceum*, when birds are bitten by infected mosquitoes, or artificially infected by injecting sporozoites obtained from salivary glands, pre-erythrocytic forms occur before the parasitemia develops from the later erythrocytic invasion. If infections are produced by inoculation of infected blood into uninfected hosts, erythrocytic forms occur first and, provided that the birds survive the initial parasitemia, EE forms appear later. Finally, if chickens are inoculated with emulsions of brains containing EE forms, a heavy EE infection results causing death before the erythrocytic development gets under way.

The relationship between erythrocytic and tissue forms is particularly interesting in *P. gallinaceum*. This species behaves differently in the three hosts, ducks, chickens and canaries, and represents different degrees of immunity. With ducks, when sporozoites (from a mosquito) are injected into the skin, abundant

cryptozoites and metacryptozoites are formed but no visible parasitemia develops, and only sub-patent parasitemia occurs. This sub-patent parasitemia may exist for up to eight months. With chickens, which are completely susceptible hosts, normal erythrocytic invasion occurs and patent parasitemia develops. With canaries, which show complete immunity, no infection of any kind develops.

The behaviour of *P. gallinaceum* in ducks then is such that it permits different kinds of parasitism to develop in the blood and in the tissues. These results have been interpreted as indicating the existence of a 'tissue-blood barrier'. Whatever the processes of immunity may be, they are unable to reach the parasites at a tissue level, and can only come into play during erythrocytic development. The effect of this is that the parasitism virtually remains at the tissue stage and the resulting infection is sub-patent.

### 8.3 Reptilian Malaria

There are probably about thirteen species of reptilian malaria parasites known but in general these have been little studied. The few species which have been investigated show characteristics similar to the avian species. The best known is *P. mexicanum*, a detailed account of which has been given by Thompson and Huff (1944). It is unusual in producing exoerythrocytic stages which correspond to the two types of avian erythrocytic stages, the *elongatum* type which occurs in a great variety of blood and blood-forming cells and the *gallinaceum* type which occurs in macrophages and true endothelial cells (p. 103). Nearly all natural infections occur in lizards of the family Iguanidae, but nothing is known of the vectors of any species.

### 8.4 Amphibian Malaria

Only one species of amphibian malaria, *P. bufonis*, from a toad, has been recognised with certainty. This species has been found in Canada but little is known of its life cycle.

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## CHAPTER IX

### SPOROZOA:

### HAEMOSPORIDEA OTHER THAN PLASMODIUM

#### 9.1 Family 2. Haemoproteidae

##### 9.11 Genus *Leucocytozoon*

*General Account.* Species of this genus are malarial-like parasites of birds which have no erythrocytic schizogony but which undergo exoerythrocytic schizogony in the parenchyma of the liver, heart or kidney. Gametocytes are found in white blood cells but not in erythrocytes. Vectors are blackflies of the genus *Simulium*, in the stomach of which development occurs. Details of some parts of the life cycle are very imperfectly known. The best known species are *Leucocytozoon simondi* (= *anatis*) in ducklings (Huff, 1942) and *L. smithi* in turkeys.

Two kinds of schizonts are found:

(a) *hepatic schizonts*—which occur mainly in the macrophages of the liver, spleen and sometimes the bone marrow. During growth, the organisms divide into a number of portions called *cytomeres*, each of which later undergoes division giving rise to merozoites.

(b) *megaschizonts*, large schizonts (60–105  $\mu$ ) which occur in the macrophages of the heart, spleen, liver and intestines.

The released merozoites penetrate *white* blood cells (lymphocytes, macrophages and monocytes) and develop into gametocytes, the earliest appearing about seven days after the initial infection. Infected leucocytes become elongated (Fig. 33) and greatly distorted and may be killed by the parasites.

The vectors are simuliid flies, the species *Simulium venustum*, *S. nigroparvum* and



FIG. 33. Gametocyte of *Leucocytozoon neavei* in erythrocyte of guinea-fowl (after Richardson, 1948).

*S. occidentale* being most commonly concerned. When infected blood is drawn into the intestine of the vector a sexual process similar to that in *Plasmodium* takes place; sporozoites pass to the salivary glands and are injected with saliva during biting.

*Synchronisation of host and parasite reproductive cycles.* It has been shown that there is a striking relationship between the degree of parasitemia with *L. simondi* and the host physiology (Chernin, 1952). During autumn and winter months (October to January) parasitemia occurs only at a low level. Almost coincidental with the onset of the sexual maturity of the bird, characterised especially by egg-laying in the female, there is a marked relapse as manifested by a high percentage of positively infected blood smears. By subjecting female birds to increased hours of artificial light per day in autumn and winter, it is possible to precipitate egg-laying weeks or even months earlier with a concomitant shift in the time of the relapse. It is thus clear that the reproductive pattern of the parasite is related to the complexity of metabolic, hormonal and anatomical changes taking place during the onset of sexual activity.

The synchronisation of reproductive patterns in host and parasite has an important zootiological significance, as the relapse parasitemia is especially characterised by a high proportion of gametocytes, thus rendering the parasite available for transmission when the adult black fly vector emerges from its overwintering larval state. Moreover when the vector begins to disseminate the infection, young and susceptible ducklings will be available.

It is tempting to conclude that the onset of the relapse and the production of gametocytes is induced by sexual hormones or their breakdown products, but this hypothesis awaits experimental confirmation. A somewhat similar synchronisation of host and parasite reproductive patterns has been shown to occur in the ciliates *Opalina* (p. 115) and *Nyctotherus* (p. 121), and the trematode *Polystoma* (p. 128). In these cases there is strong evidence for endocrinal control, and in the case of *Opalina* it has been shown that it is the level of gonadal hormones which is implicated.

### 9.12 Genus *Haemoproteus*

Species of this genus are blood parasites of birds and reptiles in which, like *Leucocytozoon*, no erythrocytic schizogony occurs. The best-known is *H. columbae* which produces a disease, sometimes fatal, with the misleading title of 'pigeon-malaria'.

*Life Cycle.* *H. columbae* occurs in the endothelial cells of the blood vessels, especially those of the lungs, liver and spleen. Within these cells, the organisms divide into cytomeres. The host cell eventually ruptures, releasing the cytomeres inside which merozoites are developing. The cytomeres disintegrate and release the merozoites, some of

which may repeat the schizogony in other endothelial cells, but others penetrate red blood cells and become male and female gametocytes. These gametocytes (Fig. 34) are shaped like a curved sausage and encircle the nucleus in a halter-fashion, and are sometimes referred to as *Halteridia*, as they were at one time called by the generic name of *Halteridium*.

The main vector is the 'pigeon fly' or 'louse fly' *Pseudolynchia canariensis*, a hippoboscid fly. The cycle in the fly is similar to that of *Leucocytozoon* or *Plasmodium*, the sporozoites settling in the salivary glands, and being transmitted during biting.

Nothing is known regarding the physiology of either *Leucocytozoon* or *Haemoproteus*.

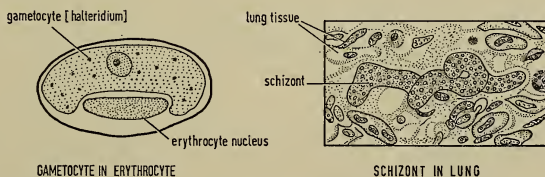


FIG. 34. *Haemoproteus columbae*—blood and tissue stages in the pigeon (after Chandler, 1955).

## 9.2 Family Babesiidae

This family includes parasites whose life cycles are very imperfectly known and some whose systematic position is uncertain.

### 9.21 Genus *Babesia*

The best known species are *B. bigemina* and *B. canis*, but about 20 species are found, mostly in the Old World. *B. bigemina* has a fairly cosmopolitan distribution but is not found in Britain; it is responsible for the disease 'red-water fever' in cattle. *B. canis* occurs in European dogs. The vectors of *B. bigemina* are one-host ticks of the genus *Boophilus*; the life cycle is shown in Fig. 35.

The trophozoites are small (2–4 $\mu$  long) oval, or pear-shaped organisms which may assume other shapes by amoeboid movement. They divide by binary fission to form two pear-shaped individuals (some species produce four) so that some red cells contain two individuals. Schizogony does not occur. There is a divergence of opinion as to the remainder of the life cycle. According to one view the products of this multiplicative phase become gametocytes which are sucked up by the tick vector and form isogametes, the zygotes forming ookinetes as in most haemosporidians. The ookinete makes its



way, not to the intestine, but to the ovary, where it penetrates the developing egg, up to 50 being found in a single egg. While the egg is developing into a larval tick, the ookinete gives rise to motile sporoblasts (termed *sporokinetes*) which ultimately give rise to sporozoites which make their way to the salivary glands of the larval tick. When the larva sucks the blood of the bovine host, sporozoites are injected in the usual manner and the life cycle is completed.

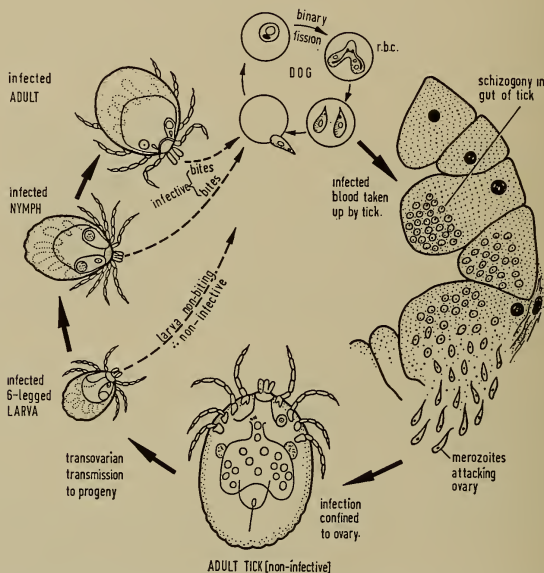


FIG. 35. *Babesia bigemina*—life cycle illustrating transovarian transmission by the one-host tick, *Boophilus* sp. (partly after Brumpt, 1949; and Richardson, 1948).

This view has been criticised, especially by German workers, who claim that the sexual and other stages in the tick have been confused with intracellular symbionts! They believe that most of the phases which reach the intestine of the female tick die off and only those entering the epithelial cells of the intestine undergo further development. Within these cells, thousands of organisms are produced which become worm-like and make their way to the ovaries and so into the eggs. The infection is thus passed from eggs to larvae and so to nymphs and finally to adults. Further fission takes place in the salivary glands of both nymphs and adults.

The older view—that is, that asexual multiplication occurs in the vertebrate host and sexual multipli-



cation in the invertebrate host—fits in well with the general pattern of the life cycles of other members of the Haemosporidea. The other view, that sexual processes do not occur in the tick, would imply that species of the genera *Babesia* (and also *Theileria*) would differ, in this respect, from all other Haemosporidea. The small size of these organisms makes interpretation of the observed stages exceedingly difficult.

Both above interpretations agree that an adult tick which has sucked up blood is not itself able to transmit the organisms because they do not penetrate to the salivary glands, as do most haemosporideans, but to the ovary. If the larva is a biting form, it will act as a vector, as will the nymph and the adult of the generation succeeding the one that became infected from the bovine host (i.e. the original vector). Knowledge of the life cycle has an important bearing on the control of the disease for it is necessary to destroy all phases of the life history for eradication to be complete. Vigorous anti-tick measures and quarantine are needed for effective control; the successful application of these measures has completely eliminated this one-time scourge of cattle from the U.S.A. The parasite is not selective about its tick host and at least thirteen species of ticks are known to transmit *B. bigemina*.

*Pathology.* The pathology of the disease produced by *Babesia* is not unlike that of malaria, involving as it does wholesale destruction of red cells. The released haemoglobin is converted into bile pigment, excess of which may be deposited in the tissues. If the liver is unable to cope with all the haemoglobin that is produced, haemoglobinuria results so that the urine is a red colour. This symptom is responsible for the name 'red-water fever' given to the disease; other names are Texas fever (U.S.A.), La Tristeza (S. America), Tick-Fever (Queensland). The term Piroplasmosis is also often used, a term derived from the older synonym *Piroplasma*.

### 9.3. Haemosporidia of Uncertain Status

#### 9.3.1 Genus *Theileria*

Only a single species, *Theileria parva*, is known. This organism is the cause of the deadly 'theileriosis' or East-Coast Fever in cattle and has a life cycle somewhat resembling that of *Babesia* except that the schizogonic stages in the vertebrate host occur, not in the red cells, but in the lymphocytes of the lymphatic system and the endothelial cells. They are most numerous in the lymphocytes of lymphatic glands and nodes. Schizogony occurs in the lymphocytes and the released bodies re-enter fresh lymphocytes. Some enter erythrocytes where they do not develop further. According to one view, these may be considered to be gametocytes which undergo development in the definitive tick host. As in the case of *Babesia*, interpretation of the developmental phases in the tick host has not been agreed on, the main point of controversy being whether or not sexual stages occur. Infective stages finally enter the salivary glands of the tick and un-

like *Babesia* do not enter the ovaries; transovarian transmission to the next generation, therefore, does not occur.

### 9.32 Genus *Toxoplasma*

This organism is a mysterious parasite of warm-blooded animals and man about which so little is known that even its animal nature is suspect, some workers considering it to be a fungus. The biology has been reviewed by Jacobs (1953).

It is believed that there is only one species, *T. gondii*, which attacks a wide range of birds and mammals. High incidences have been reported in some animal surveys: dogs 59 per cent, cats 34 per cent, pigs 30 per cent, goats 48 per cent, rats up to 20 per cent, pigeons 12 per cent. These figures are based on antibody tests which may give misleadingly high figures. In man, the reported incidence varies enormously from country to country, but exceptionally high figures, up to 68 per cent in Tahiti have been reported.

**Morphology.** The parasites are small crescent shaped cells whose size has been variously reported over a range of 3–12  $\mu$  long by 1–3  $\mu$  wide. Each end is pointed and there is a single nucleus slightly nearer one end than the other (Fig. 36). Near the nucleus is a characteristic dot—the paranuclear body. The parasites are sometimes found free in the blood stream, but more usually occur in the cells of the reticulo-endothelial system, epithelial cells or leucocytes. On injection into laboratory animals the organisms rapidly disappear from the blood stream but may be found in the spleen, liver and lungs as large 'pseudocysts' containing up to 50 organisms.



FIG. 36. *Toxoplasma gondii*, trophozoite in macrophage (after Hoare, 1949).

**Transmission.** Little is known regarding the natural method of transmission, but animals can be artificially infected by feeding with infected faeces or flesh. It is suspected that rodents or dogs may act as reservoirs for the disease.

**Pathology.** Varies from acute fatal infections to symptomless cases. In new-born infants, the disease is usually acute and rapidly fatal, being characterised particularly by the involvement of the central nervous system. From the presence of the parasites in an advanced stage at birth, it is evident that the infection is acquired *in utero* from mothers with inapparent infections. The method of transmission of the infection to the foetus is unknown. Very few adult cases have been reported.

**Cultivation.** All laboratory rodents are highly susceptible to infection so that the organism is readily maintained *in vivo*. It may also be easily maintained *in vitro* in tissue cultures of chick embryos.

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## CHAPTER X

# CILIOPHORA

This group constitutes the most highly-organised group of Protozoa and contains both free-living and parasitic species. The general morphology of the group is too well known to require detailed description.

The presence of cilia is diagnostic; many have a cytostome, often with accessory oral structures, and the majority have a macro- and micronucleus. Multiplication is by binary fission and by conjugation.

Like flagellates, ciliates require a liquid or semi-liquid environment in which to take advantage of their structural adaptations. The best-known parasitic species occur in the alimentary canal of vertebrates, especially the caecum and large intestine. The rumen and reticulum of ruminants also maintain a large ciliate fauna, some thirty-nine different species being reported from this habitat. Many invertebrates also house parasitic ciliates and species have been found in the gastrovascular cavity of medusae, the intestine of insects and annelids, the coelom and blood vessels of crustaceans, the liver of molluscs, the gonads of echinoderms and other such unusual habitats. Some are ectoparasitic. Amongst the endoparasitic species the majority are harmless, but several species are pathogenic. The group has never succeeded in becoming established in the vertebrate blood stream. The majority of ciliates are cyst-forming. Like amoebae, most stomatous ciliates are scavengers and feed on detritus; only one species, *Balanididium coli*, is a pathogen of man.

### 10.1 Classification

The sub-phylum Ciliophora is divided into two classes, Ciliata and Suctorina, but the vast majority of parasitic species are found in the former group. The Ciliata are classified as follows:

#### *Class 1. Ciliata*

*Order 1. Holotricha.* Cilia approximately equal in size with no adoral zone of flattened cilia. E.g. *Opalina*, *Ichthyophthirius*.

Order 2. *Spirotricha*. With free cilia only; exceptionally with small groups of cirrus-like projections in addition to cilia.

Sub-order 1. *Heterotricha*. Body uniformly covered with cilia. E.g. *Balantidium*, *Nyctotherus*.

Sub-order 2. *Oligotricha*. Cilia much reduced. Includes numerous species from the colon of horses and the stomach of ruminants. E.g. *Diplodinium*.

Sub-order 3. *Hypotricha*. Cirri only on ventral side. Flattened, creeping forms. E.g. *Kerona*.

Order 3. *Chonotricha*. No parasitic species.

Order 4. *Peritricha*. No parasitic species.

Class 2. *Suctoria*. Non-ciliated in the mature stage, but with ciliated free-swimming young stages. Tentacles present. A little-studied group, the majority of which are non-parasitic although some (e.g. species of *Dendrosoma*) attach themselves in enormous numbers to the gills and bodies of fresh-water crustaceans or larval arthropods.

## 10.2 Ciliata

### 10.2I Order Holotricha

Genus *Opalina*. Members of this genus are amongst the commonest and best-known parasitic ciliates. With a few exceptions, all of the some 150 known species occur in the

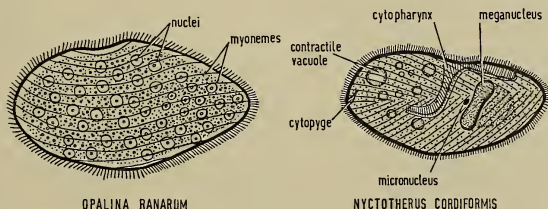


FIG. 37. *Opalina ranarum* and *Nyctotherus cordiformis* from the rectum of *Rana temporaria*.

intestine of amphibians. The best-known species are *Opalina ranarum* (Fig. 37) and *O. obtrigonoidea* from various species of frogs and toads. The body organisation is simple, and there is no cytostome. The body, which is often irregular in outline, is flattened dorso-ventrally and the pellicle is relatively tough. The size and distribution of the cilia are extremely uniform. There is a posterior 'excretory pore'. The nuclei are numerous, monomorphic, and peculiar in that in many genera they come to rest in some stage of mitosis. Many small spindle-shaped bodies of unknown character, the chromidial

# NON-BREEDING SEASON

# BREEDING SEASON

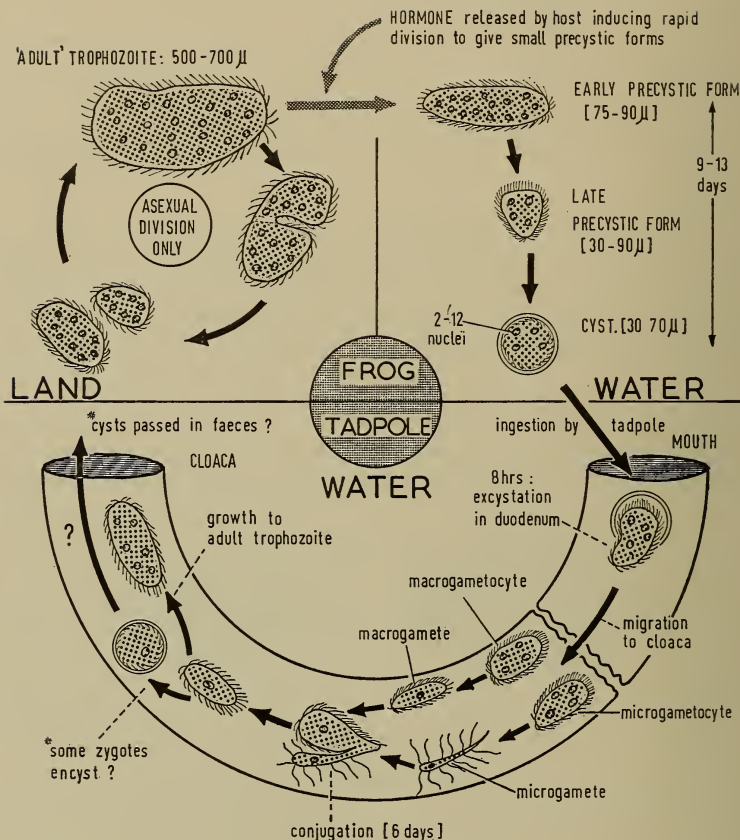


FIG. 38. *Opalina ranarum*—life cycle showing synchronisation of the cyst-producing phase of the reproductive cycle with the sexual cycle of the amphibian host. The stages marked thus \* are uncertain original; data supplied by El Mofty, 1959).









bodies, occur distributed throughout the cytoplasm. Metcalf (1909-40) has given a comprehensive account of the morphology and life cycle of ciliates of this genus. *Life cycle* (Fig. 38). The life cycle is of particular physiological interest in that its sexual reproductive phase is synchronised with that of the host and under its endocrinal control—a condition which represents a degree of metabolic dependence (p. 4) on the host more complete than in the majority of parasites. A few other parasites have a reproductive pattern similarly related to that of the host—the ciliate *Nyctotherus condiformis* (p. 120), the haemosporidean *Leucocytozoon simondi* (p. 106), various hypermastigina in insects (p. 6) and the monogenetic trematode *Polystoma integerrimum*. Although the general pattern of the life cycle is well established, small points of detail regarding the later stages of gametogeny and post-conjugation behaviour are in dispute.

During the non-breeding terrestrial phase of the amphibian host, only 'adult' trophozoites are found (Fig. 39); these divide occasionally by binary fission. As the breeding season approaches (February–March in Europe) a marked change takes place, and about fourteen days before the hosts enter the water to copulate the fission rate accelerates and small precystic forms appear. Precystic forms, which are recognisable by their size encyst to give cysts with 2–12 nuclei, four being the average number. In copulating frogs immersed in water a few cysts may be found, appearing slightly earlier in females than in males, and in both sexes cyst formation is in advance of that in *Nyctotherus* (p. 120). After ovulation and copulation are completed, the percentage of cysts rises sharply, but falls gradually later as they are passed out with the faeces. About three months after copulation cysts are no longer found. Cyst formation may be completely inhibited by enforced hibernation of potentially sexually mature frogs by maintaining them in a dry habitat (El Mofty, 1961).

Cysts are passed into the water and excyst if ingested by a tadpole, giving rise to small multinucleate forms. By further divisions, these give rise to uninucleate heterogametes which conjugate to form a zygote. According to some observations, these zygotes divide to form an 'adult' trophozoite. According to others, some zygotes encyst to form *cystozygotes* which pass out with the faeces and aid in the distribution of the species when ingested by tadpoles; *cystozygotes* excyst to form uninucleate individuals which, by repeated divisions, give rise to the normal adult trophozoites.

*In vivo endocrine control of life cycle.* It is clear from the above account that there is a change in the growth pattern of the ciliate synchronised with the breeding season of the host. It is well known that gonad maturation is under endocrinal control of gonadotrophic hormones secreted from the anterior pituitary. Injections of pregnancy urine or gonadotrophin into immature frogs induce cyst formation in *Opalina* within 9–13 days

(Bieniarz, 1950; El Mofty and Smyth, 1960). Injection of male or female hormones produced similar results, even in castrated or hypophysectomised frogs, in the period prior to the breeding season (Table 17). Testosterone propionate induced the sexual cycle during any time of the year, but oestrone was effective only prior to the breeding season. The endocrine relationships in Amphibia are complex, and it is not surprising to

		cysts
		small forms
		'adults'

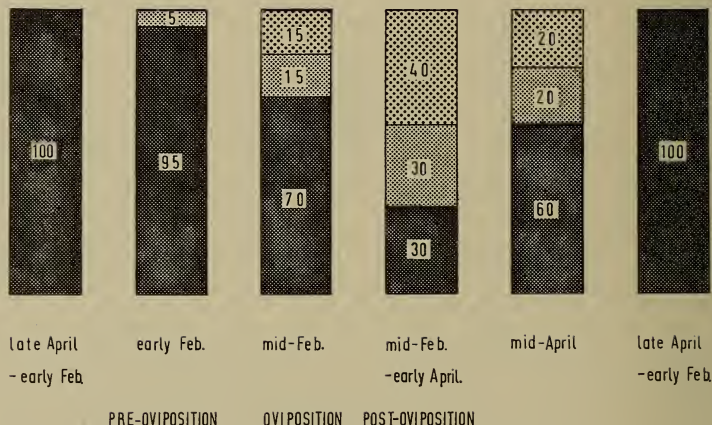


FIG. 39. Relation of cyst formation in *Opalina ranarum* to the breeding cycle of *Rana temporaria* (after El Mofty, 1959).

find that adrenaline gives the same result as testosterone propionate, that is, it induces encystation in *Opalina* during any period of the year. Just prior to the breeding season, the endocrine balance is particularly easily upset, and an increase in temperature or 'control' injections of saline can also bring about the same effect, so that experiments in this period must be interpreted with caution. Nevertheless, results with testosterone

TABLE 17

RESPONSE OF *OPALINA RANARUM* TO INJECTIONS OF VARIOUS HORMONES INTO ITS AMPHIBIAN HOST

	Pre-breeding Season			Post-breeding Season		
	Normal	Hypophysectomised	Gonadectomised	Normal	Hypophysectomised	Gonadectomised
Pregnancy urine . . . .	+	+	—	—	o	o
Chorionic gonadotrophin . .	+	+	—	—	o	o
Serum gonadotrophin . . .	+	+	—	—	o	o
Progesterone . . . . .	—	—	o	—	o	o
Oestrone . . . . .	+	+	—	—	o	o
Testosterone propionate . .	+	+	+	+	o	o
Adrenalin . . . . .	+	+	—	+	o	o

The onset of the 'sexual' reproductive cycle of the protozoan was signalled by the appearance of cysts in the rectum. + =cyst present; — =cysts absent; o=no experiment carried out. Results of experiment just prior to the breeding season must be interpreted with caution, as during this period the endocrines balance is easily disturbed and control experiments (with saline) often give positive results (data from El Mofty, 1959; El Mofty and Smyth, 1960).

propionate suggest that encystation in this protozoan is induced by the sex hormones, their excreted breakdown products, or some factor in the host induced by the presence of these substances.

*In vitro control of life cycle.* *Opalina* may be cultured *in vitro* under either non-sterile or axenic conditions using a saline-serum-albumin medium similar to that used for intestinal protozoa. Preliminary experiments (El Mofty, 1961) suggest that, *in vitro*, the organisms are less sensitive to hormones than *in vivo*. When cultured in a medium containing pregnancy urine, small forms and finally cysts appear, a result interpreted as being due to the presence in the urine of breakdown products of oestrone; cultures containing gonadotrophin failed to produce cysts. It is possible that *Opalina* and *Nyctotherus* may prove to be useful material for investigating the direct action of hormones on related compounds on cells *in vitro*.

### Genus *Ichthyophthirius*

*Ichthyophthirius multifiliis*. A well-known scourge of aquaria, causing 'white spot', a disease often fatal to fish. The ciliate has an oval body with uniform ciliation and a longitudinal striated pellicle. There is an anterior cytostome with a short cytopharynx, a large horse-shoe-shaped macro-nucleus and a small micro-nucleus. (Fig. 40). The cytoplasm is exceptionally granular and filled with large fat-like globules.

There are several hundred actively functioning contractile vacuoles, spaced evenly over the organism. It is difficult to account for this large number. Most ciliates suffice with one or two and the number and activity are less in endoparasitic forms, no doubt correlated with a lower osmotic difference. *Ichthyophthirius* is essentially an endoparasite, for it is situated *beneath* the epidermis where it forms characteristic thin-walled cysts. Within these cysts it rotates continually at a high speed, growing rapidly and giving rise

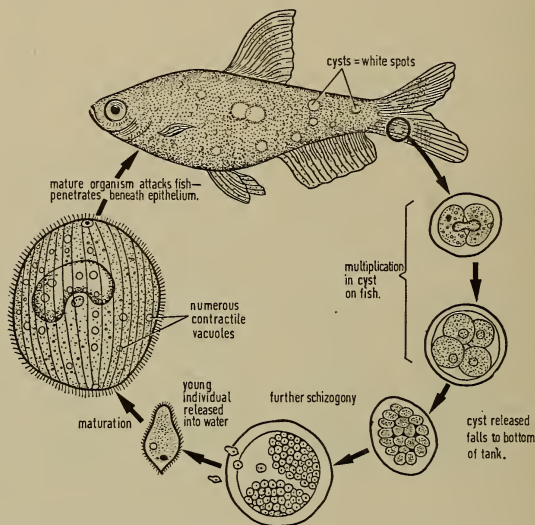


FIG. 40. *Ichthyophthirius multifiliis*—morphology and life cycle (based on several authors).

to 2-4 individuals. When these reach a certain size, they leave the host epithelium and encyst at the bottom of the aquarium. Within the cysts, a multiplication reminiscent of sporozoan schizogony takes place, the cytoplasm dividing into 100-1,000 spherical ciliated cells  $18-22\ \mu$  in diameter; these metamorphose into elongated forms measuring about  $40\ \mu \times 10\ \mu$ . The young ciliates break through the cyst wall and seek new fish hosts by actively swimming.

Nothing is known of the physiology of *Ichthyophthirius* nor has it been cultured *in vitro*.

## 10.22 Order Spirotricha

### Sub-order 1. Heterotricha

Genus *Balantidium*. This is the most studied genus of parasitic ciliates, for it includes the pathogenic species *B. coli*. The majority of species occur in the large intestine of vertebrates, but species are also found in invertebrates.

*Balantidium coli* (Fig. 41). This is the largest protozoan parasite of man, with a size

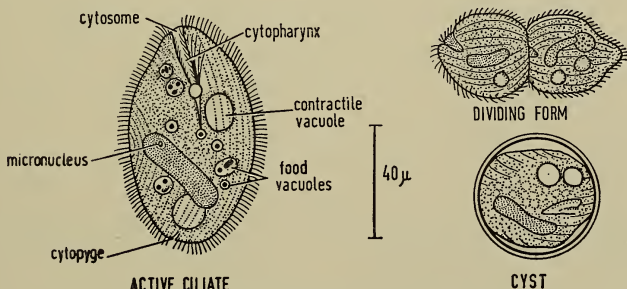


FIG. 41. *Balantidium coli* (adapted from Hoare, 1949).

variously reported in the range 30–200  $\mu$  long, by 20–120  $\mu$  in breadth; the upper limits referring to specimens from pigs.

The general outline is egg-shaped, bearing an anterior slit-like peristome opening into a cytostome. The whole body is covered with small fine cilia with an adoral zone around the peristome. There is a well-marked macronucleus, and inconspicuous micronucleus, and two contractile vacuoles. Food vacuoles are commonly present. Reproduction is by binary fission and conjugation, as in the majority of heterotrich ciliates.

The main animal reservoir of the species is probably the pig, but the organism also occurs in monkeys and rats. In some parts of Britain, the incidence in pigs is almost 100 per cent but in man it is relatively rare. In both pigs and man, balantidia live in the caecum or colon, feeding on intestinal contents, especially bacteria, without penetration of the tissues. In pigs, it is non-pathogenic. In man, it may be harmless, but under circumstances not understood it may, like *Entamoeba histolytica*, invade the mucosa and sub-mucosa in large numbers and feed on red blood corpuscles causing *balantidial dysentery*, characterised by ulceration of the infected area. Severe infections may be fatal.

Under certain (unknown) conditions, encystment takes place and cysts are passed



in the faeces. Infection is by direct ingestion of cysts, but large or repeated doses are necessary to establish infection in man.

It was formerly thought that another species, *B. suis*, occurred in pigs. Specimens of this species were described as being smaller than *B. coli* with a size range of 35–120  $\mu$  by 20–60  $\mu$ . *In vitro* studies have recently shown that the so-called trophozoites of *B. suis* are actually pre-conjugants of *B. coli*.

*In vitro cultivation.* As *Balantidium* is essentially an intestinal scavenger, it is not surprising to find that it may be cultured *in vitro* under the conditions similar to those found suitable for species of *Entamoeba* and other intestinal protozoans, i.e. a diphasic medium of coagulated horse-serum slope-covered with diluted serum with added rich starch (p. 159). *In vitro*, at 37° C., ciliates conjugate regularly, but at 25° C. they cease to conjugate after about a month. Little is known of the physiology of *Balantidium*, except that it can tolerate a fairly wide range of oxygen tensions.

*Other species of Balantidia.* Numerous species occur in frogs and salamanders, e.g. *B. entozoon*, *B. rotundum*, *B. gracilis*. Other species available for laboratory study are *B. blattarum* in cockroaches, *B. pisciola* in fishes, and *B. caviae* in guinea pigs.

*Genus Nyctotherus.* Species of this genus are amongst the best-known of the protozoan parasites of frogs and toads.

*N. cordiformis* (Fig. 37). Occurs in the rectum of frogs and tadpoles. It is completely ciliated with a well-marked curved peristome leading to a cytostome, and a cytopharynx. There is a kidney-shaped macronucleus, a small micronucleus, a posterior contractile vacuole, and an excretory pore.

*Life cycle* (Fig. 42). The reproductive cycle of *Nyctotherus*, like that of *Opalina* (p. 115), is linked with the sexual cycle of the amphibian host. During the year, *Nyctotherus* divides occasionally by binary fission. In spring, parallel with the frog breeding season, a change from asexual to sexual multiplication takes place, and trophozoites divide with greater frequency, finally forming mononuclear precystic forms. These encyst and pass out into the water with the host faeces. The cysts are ingested by tadpoles developed from eggs laid in the same water, and the young individuals, the pre-conjugants, hatch out. These conjugate and undergo a nuclear process similar to the well-known process in *Paramoecium*. The conjugants separate and the ex-conjugants, which occur almost exclusively in recently metamorphosed frogs, undergo repeated binary fission as before (El Mofty, 1961; Bieniarz, 1950).

Preliminary experiments on frogs have given similar results to those already described for *Opalina ranarum* (p. 115). When pregnant human urine or male or female hormones are injected into frogs under certain conditions, encystation of *Nyctotherus*



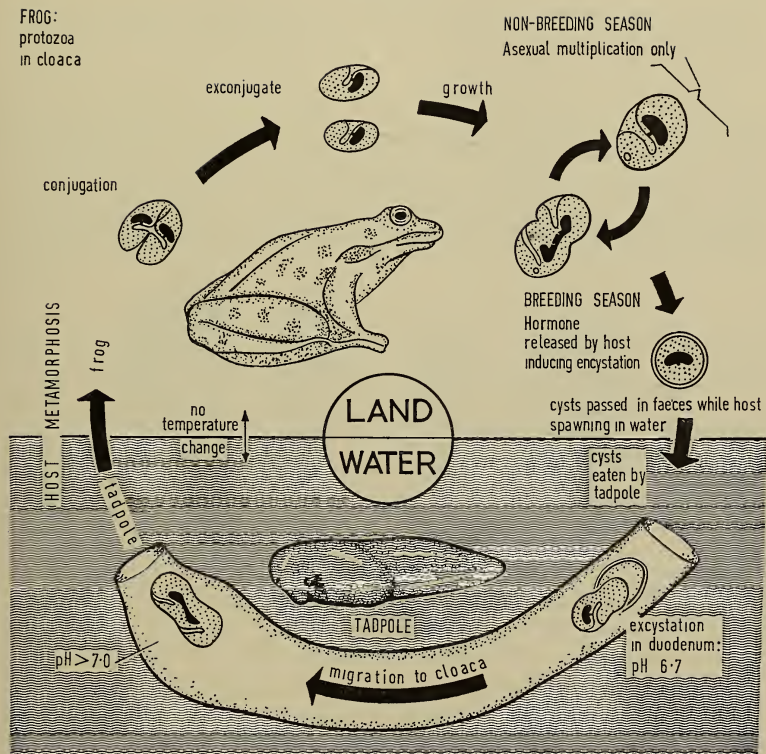


FIG. 42. *Nyctotherus cordiformis*—life cycle showing relation to breeding cycle of *Rana temporaria* (original).

takes place, suggesting that, as in the case of *Opalina*, the process is related to the level of the gonadal hormones in the host.

#### Sub-order 2. *Oligotricha*.

Symbiotic oligotrichs occur in quantity in ungulates and rodents; none occurs in carnivorous animals. The most favoured sites are the rumen and reticulum of cattle which may contain anything up to thirty-nine different species of ciliates. These belong

mainly to the genera *Isotricha*, *Bütschlia*, *Entodinium* and *Diplodinium*. Their morphology ranges from relatively simple species such as *Bütschlia* to *Diplodinium* (Fig. 43), the most complicated protozoan known.

Rumen ciliates are particularly interesting on account of the role they play in digestion in cattle. That they were essentially symbiotic protozoans was long suspected but it was not until the presence of a cellulase, active in the pH range 4.0-6.6, was found in *Eudiplodinium neglectum* that convincing evidence was forthcoming. A cellulase and a cellobiase have also been detected in *Diplodinium* but not in *Entodinium*, *Isotricha*, *Dasytricha* or *Bütschlia*. Presumably *Entodinium* absorbs carbohydrate from the hydrolysis

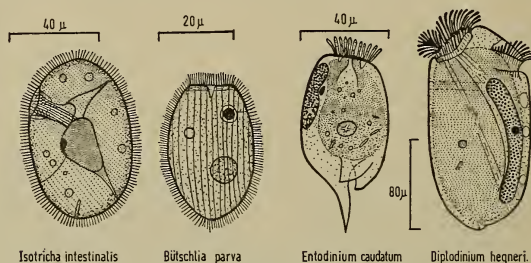


FIG. 43. Ciliates from the rumen of cattle (after Hegner *et al.*, 1938).

of cellulose through bacterial action. *Entodinium* is morphologically considered to be more primitive than *Diplodinium* and the latter is believed to have been derived from the former. If this be so, *Entodinium* has lost its power of synthesising cellulose.

The metabolism of these ciliates is intense and they can multiply very rapidly although their life period is only about twenty-four hours. Consequently, they are continually dying and disintegrating. This provides the host with a substantial proportion of its nitrogen and carbohydrate requirements. Ruminants which have been de-faunated are only able to utilise cellulose if sufficient cellulose-splitting bacteria are present to replace the ciliates. The host is thus metabolically dependent on these organisms to a considerable degree, and the relationship between these two organisms represents a classical example of the mutual metabolic dependence inherent in certain parasitic associations (p. 7).

Rumen ciliates are obligate anaerobes and are killed by oxygen but may be cultured in mixtures of rumen fluid, dried grass and cellulose in an atmosphere of 95 per cent  $N_2$  and 5 per cent  $CO_2$ . Little is known of their growth requirements

except that an unknown factor present in rumen fluid is essential. Their physiology has been reviewed by Oxford (1955).

*Sub-order 3. Hypotricha.*

An example of this sub-order is the common species *Kerona pediculus* which occurs on the fresh-water coelenterate *Hydra*, especially *H. fusca* and *H. vulgaris*. It is a true ectoparasite feeding on the living cells of the *Hydra*, which it may ultimately destroy. It has almost the same structure as the typical free-living hypotrich, but is especially adapted for gliding over the surface of *Hydra*; its anterior and lateral edges are flexible.

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## CHAPTER XI

# HELMINTH PARASITES: TREMATODA – MONOGENEA

The term 'worms' is loosely applied to an assemblage of organisms with elongated bodies and a more or less creeping habit. The term has no precise zoological meaning but is retained as a convenient operational word for defining a kind of organism well recognised but precisely indefinable. The term *helminth* (derived from the Greek words *helmins* or *helminthos*), although literally also meaning 'worm', zoologically speaking has a more precise connotation and is nowadays restricted to members of the Phyla Platyhelminthes, Nematoda and Acanthocephala. Although the former phylum includes the free-living turbellarians, the study of helminths—or helminthology—has come to be regarded as being confined to the study of parasitic forms. Helminths typically parasitise vertebrates, although invertebrates, especially arthropods and molluscs, act as intermediate hosts.

Helminths may be classified as follows:

*Phylum Platyhelminthes*. Body dorso-ventrally flattened, bilaterally symmetrical, without definite anus, body cavity lacking. Organs embedded in specialised connective tissue known as parenchyma. Usually hermaphrodite. Respiratory and circulatory systems absent, flame cells in excretory system.

*Class 1. Turbellaria*. Fresh-water, marine or terrestrial platyhelminthes with ciliated epidermis. Almost entirely free-living.

*Class 2. Trematoda* (flukes). Entirely ecto- or endoparasites, devoid of ciliated epidermis, covered with cuticle, body undivided, well developed adhesive organs and alimentary canal.

*Class 3. Cestoda* (tapeworms). Elongated endoparasites with alimentary canal lacking, devoid of ciliated epidermis, usually divided into segments, adhesive organs at anterior end; in adult stage parasites of alimentary canal of vertebrates, life cycle complicated with two or more hosts.

*Phylum Nematoda* (roundworms). Unsegmented, cylindrical worms with a resistant cuticle, devoid of cilia. Sexes separate, gonads tubular and continuous with their ducts. Body cavity a pseudocoel (p. 302). Free-living and parasitic.

*Phylum Nematomorpha* (hairworms). Unsegmented, long thread-like free-living worms occurring in soil or water. Sexes separate, adults free-living, larvae parasitic in arthropods (not considered here).

*Phylum Acanthocephala* (spiney-headed worms). Unsegmented, cylindrical worms with an armed protrusible proboscis. Pseudocoel, alimentary canal lacking. Sexes separate, parasitic in the adult stage in intestine of vertebrates.

### II.1 Trematoda—Introduction

The term Trematoda is derived from the Greek *trematodes* (having holes), referring to the suckers which form a characteristic feature of the group. Trematodes occur in a wide range of host environments; the majority of species are endoparasitic but many are ectoparasitic. The larval stages may occur in invertebrate (especially mollusc) hosts and vertebrate hosts, but the majority of adult stages occur in or on vertebrates. Specimens range in size from 1 mm to 75 mm and are usually grey or creamy white in colour, but many acquire a characteristic coloration due to the assimilation of food materials from the host. The mouth and reproductive apertures are typically anterior. The digestive system is well developed and food material consists of intestinal debris, blood, mucus or other tissue exudants depending on the nature of the host environment. They fall readily into three groups: the *Monogenea*—typically external parasites of fish with direct life cycles; the *Aspidogastrea*—endoparasitic species with the entire ventral surface an adhesive organ; and the *Digenea*—endoparasitic species with simpler adhesive organs and indirect life cycle. A more detailed description of each order is as follows: *Order 1. Monogenea*. Adhesive organs consisting of an *opisthaptor*, a posterior disc which may bear suckers or hooks or both, and a *prohaptor* usually in the form of an oral sucker, often absent or poorly developed. Paired excretory pores, anterior separate and dorsal. Parasites of the skin and other superficial locations, especially on the gills of fishes. Life cycle simple, no alteration of hosts.

*Order 2. Aspidogastrea*. Main adhesive organ occupying almost the entire ventral surface of the body and consisting of suckerlets arranged in rows. No hooks, oral sucker absent, excretory pore, single and posterior. Endoparasites of vertebrates (especially chelonians and fishes), mollusca and crustacea. Life cycles, as far as known, simple.

*Order 3. Digenea*. Main adhesive organ a single ventral sucker. Oral sucker present. Excretory pore single and posterior (contrast *Monogenea*). Larval stages invariably in

molluscs (two reported from annelids). With rare exceptions, endoparasites of vertebrates in the adult stage.

## II.2 Order I. Monogenea

### II.2I Type Example: *Polystoma integerrimum*

This species is a parasite of the excretory bladder of European frogs. Although common, it frequently has a markedly local distribution. The name *Polystoma* is

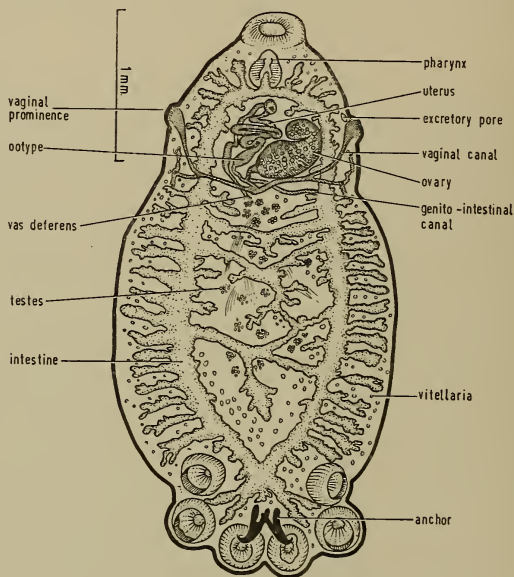


FIG. 44. *Polystoma integerrimum*—normal adult from bladder of frog. Genital organs become mature only during breeding season of host (from an original drawing by Miss J. B. Williams).

derived from an observer who mistook the posterior suckers for mouths. Its anatomy has been reinvestigated by Williams (1957).

*External features.* The most striking feature (Fig. 44) is the opisthaptor, an apparatus consisting of six posterior suckers arranged in a circlet on a posterior muscular disc.



Two hooks lie slightly anterior to the most posterior pair of suckers. The anterior adhesive organ or *prohaptor* takes the form of an oral sucker surrounding the terminal mouth. The male and uterine openings form a common genital opening in the mid-ventral line. Two marginal swellings at the anterior end indicate the positions of the two vaginal openings.

*Body wall.* The body is covered by a plicated cuticle beneath which is a hypodermis and three muscle layers (outer circular, oblique and inner longitudinal). As in other platyhelminths, the internal spaces between the organs are filled with a mesenchymatous parenchyma made up of typical polygonal cells.

*Digestive system.* The mouth leads into a short muscular pharynx leading to the intestine. A peculiar median passage, the *bucco-intestinal* canal, extends forward beneath the pharynx and oesophagus and communicates with the buccal tube. This forms an alternative passage between the oral cavity and the intestine. The intestine divides into two branches which bear lateral caeca and those from each branch may anastomose so that a widespread system is formed. The parasite feeds on blood and the outline of the gut is often seen to stand out clearly with contained blood. Nothing is known of the enzymes concerned in digestion.

*Excretory system.* A typical platyhelminth protonephridial system is present. Flame cells lead to fine canals which join to form paired excretory canals opening anteriorly and dorsally at about the same level of the pharynx. The excretory material contains haematin which is passed in the host's urine. The presence of this pigment may be used to detect the presence of *Polystoma* in frogs (p. 131).

*Nervous system.* This consists of a nerving surrounding the pharynx from which nerves extend anteriorly and posteriorly. The muscles of the opisthaptor are supplied with fibres from the ventral nerves.

*Reproductive system.* Male: In general, this resembles that of rhabdocoele turbellarians. The testes are scattered with fine ducts connecting to a penis leading to the median genital pore.

Female: The oviduct arises from the single ovary. Shortly after its origin there opens into it (a) a *genito-intestinal canal* which leads to the intestine and (b) a short median vitelline duct. The latter bifurcates to give transverse vitelline ducts which run anteriorly and posteriorly. Into the anterior ducts are connected a pair of vaginae which open by small pores at marginal vaginal swellings. The oviduct passes forward to Mehlis' gland, and so to the uterus.

Thus, in *Polystoma*, the vaginae enter the vitelline ducts and into the oviduct, as in many Monogenea; hence the sperms enter the oviduct with the yolk. The occurrence

of a genito-intestinal canal is not a general feature of Monogenea, but only occurs in the *Polyophisthocotylea*. It also occurs in some triclad, and may be a relic of the time when, in some primitive species (e.g. *Acoela*) the ova escaped from the parenchyma into the gut and out through the mouth. It has also been suggested that it could act as an escape arrangement for surplus yolk and ova.

The egg-shell is formed in the same way as in the digenetic trematodes and pseudophyllidean cestodes by the release of large semi-liquid globules from the so-called

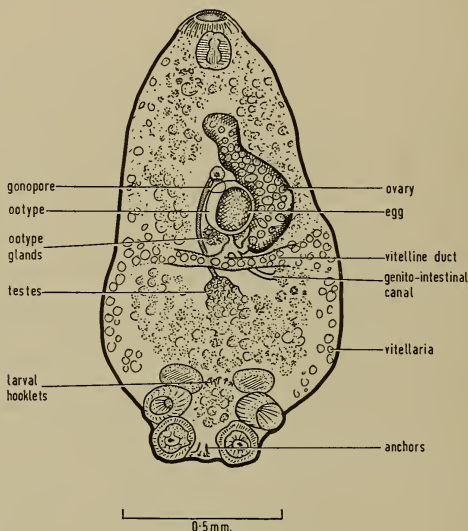


FIG. 45. *Polystoma integerrimum*—neotenic adult from external gills of frog tadpole (from an original drawing by Miss J. B. Williams).

vitelline cells. These run together and form a shell which is probably a tanned protein as in the Digenea (p. 144).

*Life cycle.* The life cycle of *Polystoma* (Fig. 46) is one of exceptional interest as being the only known helminth parasite whose maturation bears a clear relationship to sexual maturation of its host. In spring when the frog is preparing to enter the water preparatory to copulation, the genitalia of the fluke begin to ripen. When frogs actually enter

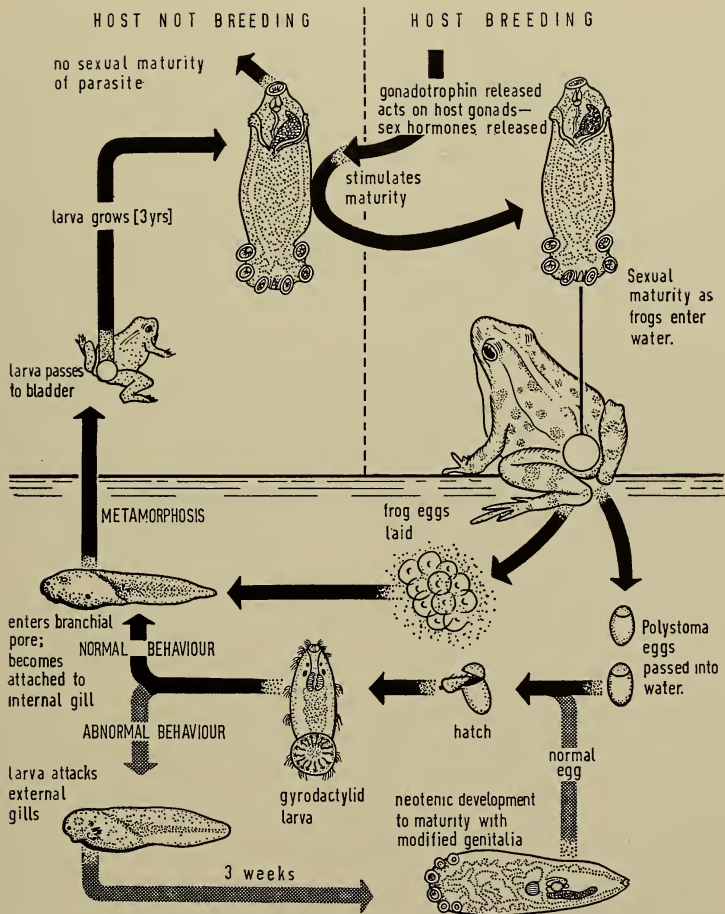


FIG. 46. Life cycle of *Polystoma integerrimum*, showing synchronisation of sexual maturity with breeding cycle of amphibian host (original; based on the work of Miretski, 1951).

the water, maturation of *Polystoma* is completed and large quantities of eggs are released. These eggs hatch in about the same time as that required for the eggs produced by the host frogs to reach the internal gill tadpole stage. The hatched larva or *gyrodactylid larva* is barrel-shaped with a large posterior sucker bearing sixteen hooks, a primitive gut, and five incomplete bands of cilia. This larva makes its way into the branchial pore of the tadpole and attaches itself to the gills where it feeds on mucus and detritus. When the tadpole metamorphoses into a young frog, the larva passes down the digestive tract and eventually reaches the bladder where it becomes attached. During this migration it loses its cilia and develops the six-suckered opisthaptor characteristic of the adult. Maturation in the bladder is unusually slow, even for a cold-blooded parasite, and requires three years.

The cycle described above is probably the most commonly enacted one, but an alternative cycle can occur, if, by chance, a larva becomes attached to the *external* gills of a tadpole. In this case, rapid neotenic development takes place and a miniature sexually mature fluke is formed within about twenty days. The neotenic adult (Fig. 45) differs greatly from the normal adult matured in the bladder, having only a single testis, functionless copulatory organs, headless sperms, and rudimentary uterus, vaginae and vitellaria. Cross-fertilisation takes place although the definitive sperm is only a late spermatid. Notwithstanding these extraordinary morphological abnormalities, neotenic adults developing in this way give rise to fertile eggs which hatch to produce larvae which undergo normal development.

Recent experiments (Miretski, 1951) have revealed that the maturity of the fluke is probably controlled directly or indirectly by the hormonal activity of the frog. Thus when infected immature frogs are injected with hypophysis extract, the polystomes mature within four to eight days and produce quantities of eggs for a period of about a week. This latter time corresponds approximately to the time frogs spend on spawning. After this period, no further eggs are produced. This beautifully synchronised mechanism results in the eggs of the fluke being released only when the frogs enter water to breed, thus assuring that by the time they have passed through the embryo stage and hatched, abundant tadpoles will be available for re-infection. The nature of the endocrinal control has not been established. Injection of hypophysis extract would result in increased levels of gonadotrophins and gonadal hormones, either of which could be responsible for the maturation effect. In the case of *Opalina* and *Nyctotherus* (p. 120), whose life cycles are similarly synchronised with that of the host, there is evidence that the gonadal hormones are directly or indirectly responsible; the same mechanism is likely to operate for *Polystoma*.

*Detection of Polystoma in frogs.* Jennings (1956) has developed a technique for the detection of *Polystoma integerrimum* in frogs based on the presence of haematin in the urine of infected hosts. By gently squeezing a frog a little urine is collected into a 2½-in. × ¼-in. tube to which is added an alkaline solution of luminol and hydrogen peroxide. The presence of haematin, and hence *Polystoma*, is indicated by the development of an intense blue luminescence. In carrying out this test, faecal contamination must be avoided, as the peroxidases of the faecal protozoa act with the luminol-peroxide reagent to give a false positive reaction.

## 11.22 General Account

*Distribution.* The majority of species are parasitic on fish, and of these a high proportion occur on the gills. A list of common species occurring in some European marine fish is given in Table 18. In many cases, the organisms show a marked predilection for the particular gill or gills. Thus, *Diclidophora merlangi* (Fig. 47) occurs most frequently on

TABLE 18  
OCCURRENCE OF MONOGENETIC TREMATODES ON FISH  
EXAMINED AT PLYMOUTH, ENGLAND, DURING 1953-55  
(data from Llewellyn, 1956)

Host	Parasite	Per cent infection
<i>Gadus merlangus</i> (whiting)	<i>Diclidophora merlangi</i>	8.7
<i>Gadus luscus</i> (pout, bib)	<i>Diclidophora luscae</i>	21.0
<i>Merluccius merluccius</i> (hake)	<i>Anthocotyle merlucci</i>	7.6
<i>Trigla cuculus</i> (red gurnard)	<i>Plectanocotyle gurnardi</i>	95.0
<i>Trachurus trachurus</i> (horse mackerel)	<i>Pseudaxine trachuri</i>	21.6
<i>Trachurus trachurus</i> (horse mackerel)	<i>Gastrocotyle trachuri</i>	62.2
<i>Belone belone</i> (gar fish)	<i>Axine belones</i>	77.8
<i>Morone labrax</i> (bass)	<i>Microcotyle labracis</i>	66.7
<i>Scomber scombrus</i> (mackerel)	<i>Kuhnia scomбри</i>	100.0

the first gill of the whiting, whereas *D. luscae* occurs on the second and third gill of the pout, and other similar cases of predilection are known. All gill-inhabiting species take up the same general adhesive attitude with the posterior organs nearer to the gill arch of the host and the anterior end nearer to the distal end of the primary lamellae (Llewellyn, 1956); the mouth-bearing end thus faces downwards.

*Nutrition.* Species inhabiting the gills, buccal cavity and bladder feed mainly on blood, and often produce a black pigment as a breakdown product from the haemoglobin. Cloacal forms feed on mucus. Nothing is known regarding the nature of the digestive enzymes.

*Reproduction.* The male system in general shows little variation from that described for *Polystoma*. The testes may consist of a solid body or scattered capsules. The penis is

armed in some species and possesses so-called prostatic glands. The ovary is usually elongate and the vitellaria follicular. The ootype region may contain other gland cells as well as those of Mehlis' gland, but their role in capsule formation is unknown. The

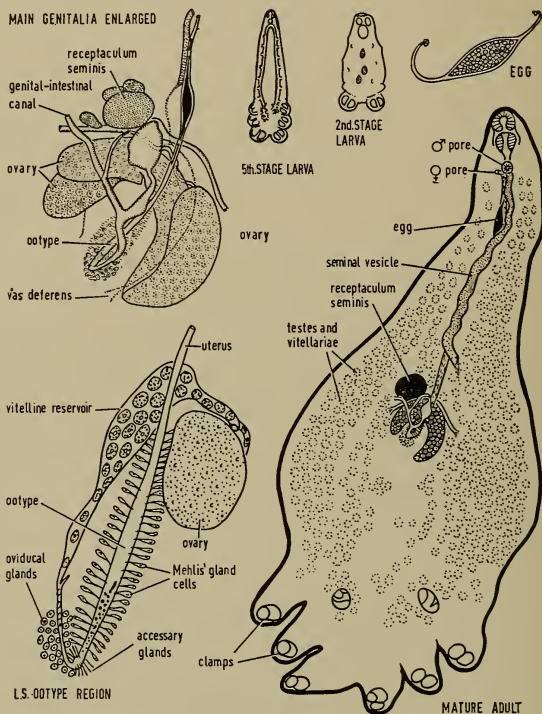


FIG. 47. *Dichidophora merlangi*—anatomy of adult and larvae (from Rennison, 1953; and Frankland, 1955).

eggs are always operculate and frequently bear long filaments at one or both ends (Fig. 47). The egg-capsules are yellow or brown and probably consist of a quinone-tanned protein as in many digenetic forms. The mechanism of egg-capsule formation closely resembles that of the digenetic trematodes (p. 143) and the pseudophyllidean cestodes



(p. 241). It is interesting to note that in the viviparous form *Gyrodactylus* (Fig. 48) vitellaria are lacking, the ovary being a germovitelarium (i.e. an ovary with specialised yolk-producing regions).

### 11.23 Miscellaneous Monogenea

*Gyrodactylus elegans* (Fig. 48). This is a small ectoparasite of fresh-water and marine fish, often easily obtainable from aquaria fish. It is unusual in that it is not only viviparous but the larva forming in the uterus of the parent may contain a further embryo which itself contains a mass of embryonic cells, the whole forming a kind of Chinese

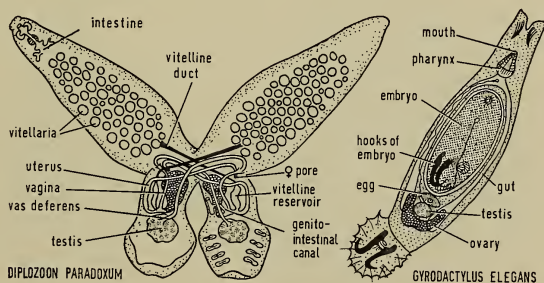


FIG. 48. Two aberrant monogenetic trematodes. *Diplozoon paradoxum*, from the gills of the minnow; *Gyrodactylus elegans*, from the skin of aquarium fish (after Dawes, 1946).

box of developing forms. Up to four generations may occur in one individual. This is a phenomenon not understood. Polyembryology may be ruled out, for if all the larvae were originally from the same eggs, they would have all been the same age, unless some differential factor was operating.

*Calicotyle kroyeri* (Fig. 49). A not uncommon species occurring in the cloacal region of skate or ray. It is a squat form almost as broad as long. The opisthaptor is sucker-like with seven radial septa and two hooks. The vaginae are paired and open ventrolaterally slightly behind the level of the genital aperture. Eggs are not numerous.

*Polystomoides oris*. A species closely related to *Polystoma integerrimum*; parasitic in the mouth cavity of the fresh-water painted turtle, *Chrysemys picta*. Its basic morphology is similar to that of *Polystoma* but its life cycle is simpler, bearing no relation to the breeding cycle of the host. Eggs hatch at laboratory temperatures in about a month, and the hatched larvae enter the mouth of other turtles and reach maturity in about a year.

*Diclidophora merlangi* (Fig. 47). This species is a common parasite on the gills of whiting (*Gadus merlangus*) and probably the most easily obtainable monogenetic trematode suitable for class material. Unfortunately, as its branched gut is usually filled with pigment, derived from its blood meals, the general morphology is difficult to follow in whole mounts. It lacks vaginae and has an elongated folded ovary. The opisthaptor consists of four pairs of pedunculate clamp-like suckers each bearing a complex cuticular frame-

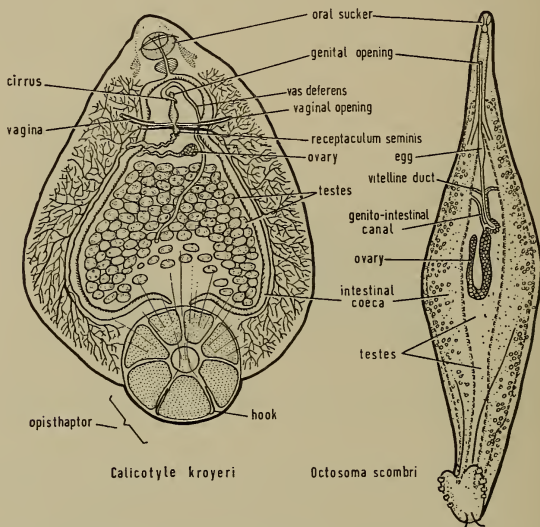


FIG. 49. Two common monogenetic trematodes. *Calicotyle kroyeri*, from cloaca of skates and rays; *Kuhnia scombr*, from gills of mackerels (after Goto, 1894).

work of sclerites. The mouth is not quite terminal and the gut branches extensively into the peduncles of the suckers.

*Diplozoon paradoxum* (Fig. 48). A remarkable species from the gills of the minnow and comprising two individuals which become completely fused to one another. The attachment takes place in the larval stage, a pair being held together by a sucker arrangement which finally disappears as the organisms become fused together. When mature, the vagina of one individual opens opposite the openings of the uterus and vas deferens

of the other so that cross-fertilisation is possible and may be the rule. Larvae which fail to find a partner subsequently die and never attain sexual maturity. A physiological explanation for the failure of single individuals to become mature has not yet been produced.

*Kuhnia scombri* (Fig. 49). A common parasite of the gills of mackerel, infections of up to 100 per cent occurring in some areas off the coast of Great Britain (Table 18). General morphology resembles *Diclidophora* but a vaginal opening is present.

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## CHAPTER XII

### TREMATODA: ASPIDOGASTREA

This order contains only the single family Aspidogastridae with nine genera. They are all parasites of poikilothermic animals, fishes, chelonians, molluscs and crustaceans. Although the morphology of a number of species is well known (Bychowsky and Bychowsky, 1934), knowledge of the physiology and life cycle of the group is scanty.

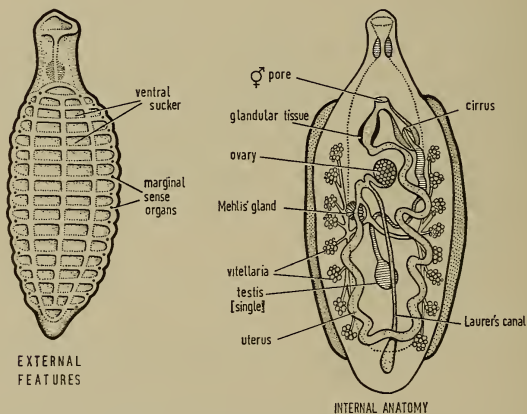


FIG. 50. *Aspidogastrea* (after Monticelli and Lankester).

The best known life cycle is that of *Aspidogaster conchicola*, parasitic in the fresh-water mussels *Anodonta* and *Unio* (Williams, 1942).

*External features.* The most striking external feature is the enormous adhesive apparatus which occupies almost the entire ventral surface. This organ is made up of numerous compartments or alveoli, each of which can act as an efficient sucker (Fig. 50).

*Body wall.* The structure of this is more complicated than in other trematodes. It consists of a cuticle, beneath which is a thin layer of circular muscles, a layer of hollow longitudinal muscles, two intersecting layers of diagonal muscles, and finally a further layer of circular muscles.

*General internal features.* As in other trematodes, the internal organs are supported by loose parenchyma. In the *Aspidogastrea*, however, a unique *septum*, not found in other trematodes, occurs. This divides the body into upper and lower compartments, the upper containing the alimentary canal, the terminal genital ducts and the vitellaria, and the lower the ovary, oviduct, ootype and testes.

*Alimentary canal.* The mouth opens into a muscular pharynx and leads to a sac-like intestine. Nothing is known of the enzyme system concerned in digestion.

*Excretory system.* This consists of a flame-cell system with complicated branching. There are two main lateral excretory canals which open posteriorly by two funnel-like pores.

*Reproductive system.* The reproductive system has the typical trematode pattern arranged as shown in Fig. 50, but with an unusual feature in the elongate blind Laurer's canal. Both self-fertilisation and cross-fertilisation have been observed. The egg-shell material is produced by the vitelline cells, as in other trematodes, but the nature of the egg-shell has not been investigated.

*Life cycle.* Larvae develop within the eggs while still *in utero*, and hatch out within the same host or in water. If an infected mussel is eaten by a fish, the adults may attach themselves to the stomach or intestine of the host and initiate a second-host association. This group thus provides evidence indicating how alternation of hosts is in process of being achieved apparently without alternation of generations. This position could be interpreted as being intermediate between Monogenea and Digenea in this respect.

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## CHAPTER XIII

# TREMATODA: DIGENEA

The great majority of digenetic trematodes are inhabitants of the vertebrate alimentary canal or its associated organs, especially the liver, bile duct, gall bladder, lungs, pancreatic duct, ureter and bladder. These are organs containing cavities rich in potential semi-solid food materials such as blood, bile, mucus and intestinal debris. The digenetic trematodes are readily distinguished microscopically from the other trematodes by their relatively simple external structure, in particular the absence of complicated adhesive organs; only simple suckers are present. They also differ markedly from the other trematode orders in having complex heteroxenous life cycles involving at least one intermediate host; from this feature the term *Digenea* is derived.

With two exceptions, in all life cycles the first intermediate host is a mollusc, usually a gastropod, occasionally a lamellibranch or a scaphopod but never a member of any other molluscan order. In the exceptional cases, the first intermediate host is an annelid. The larval phases are unusual in undergoing polyembryology so that enormous numbers of larvae may result from small initial infections.

### 13.1 General Morphology

The morphology of a 'generalised' fluke is given in Fig. 51, to show the relationship between the various organs.

*External features.* Most species are flattened dorso-ventrally but some have thick fleshy bodies and some are round in section. There are typically two suckers, an anterior *oral* sucker surrounding the mouth at the anterior end, and a *ventral* sucker on the ventral surface.

*Body wall.* The cuticle is strong yet elastic and usually rests on a definite basement membrane. The origin of the cuticle is obscure, as there is no distinct epithelium. There are generally three layers of muscles: an outer circular, a middle diagonal and an inner longitudinal. An extra layer of circular muscles is occasionally present, as are vertical muscles transversing the parenchyma. The muscle fibres are apparently un-



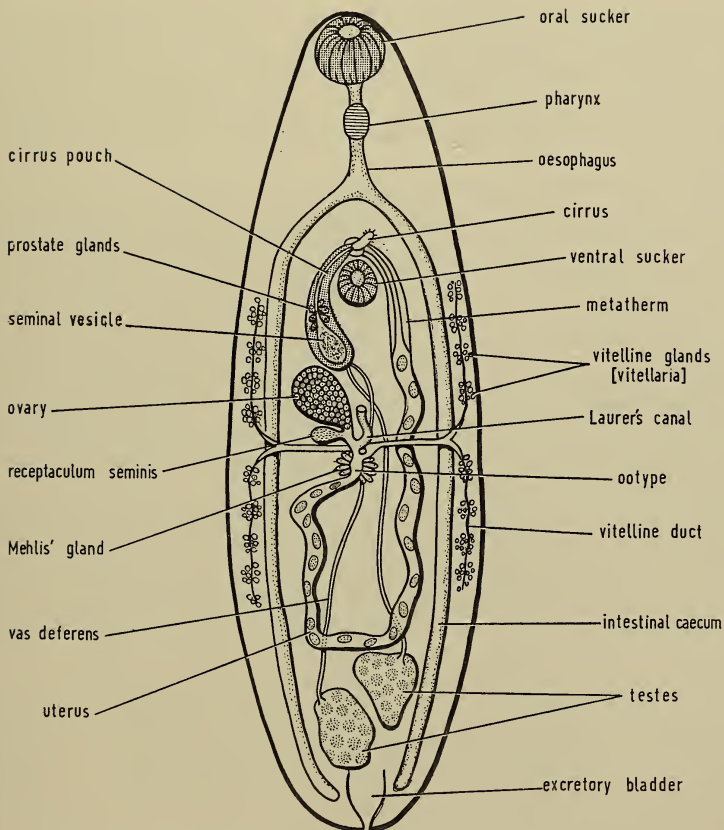


FIG. 51. A generalised diagram of trematode anatomy (modified from Cable, 1949).

striated. The tissue framework of the body is made up of parenchyma, a network of cells and fibres enclosing irregular spaces.

*Nervous system.* The nervous system is relatively simple and consists of paired cerebral ganglia and a submuscular plexus in the form of longitudinal cords and transverse

connectives. The cerebral ganglia are connected by a broad commissure. Three pairs of nerves pass anteriorly from the ganglia and three pairs pass posteriorly, and in the latter group the ventral pair are especially well developed. Various fibrils arise from these nerves to various parts of the body. Most of these fibrils are motor, the sensory elements being feebly developed. Eye spots occur in some larval forms, particularly miracidia.

*Digestive system.* The alimentary canal is usually well developed with a prominent pharynx and oesophagus. In the gasterostomes (Figs. 59 and 60) it is a simple blind sac, but in the majority of species it consists of branched caeca which occasionally branch further (e.g. *Fasciola*). The caeca may reach to the posterior end or only halfway down the body. Trematodes lack an anus, except in two genera (*Diploproctodaeum* and *Opecoelus*).

Little is known of the enzyme system of the alimentary canal and the common digestive enzymes have not been identified. It is generally stated that the food of intestinal trematodes consists of semi-digested food. Recent *in vitro* work suggests that trematodes can digest complex proteinaceous materials such as albumen, and that those in the bile ducts or lungs live mainly on blood and mucus. The nutrition of trematodes is further discussed on p. 214.

*Excretory system.* This is essentially a protonephridial one, similar to that of turbellarians with the flame cell as the excretory unit. The arrangement of the flame cells, i.e. their number and manner of branching of their ducts, are of systematic importance and often of diagnostic value, especially in larvae (Faust, 1932; Stunkard, 1946; La Rue, 1957). Although the arrangement differs in different families, it is similar in closely related species.

The basic flame-cell pattern is most readily studied in the cercarial stage where it is not masked by opaque tissues. As a cercaria develops to an adult fluke, the excretory system which is formed is an exact multiple of the simple cercarial type. Thus in the cercaria of the blood flukes of the genus *Schistosoma*, there are an anterior and posterior pair of nephridia on each side of the body (Fig. 52) and this arrangement is given the following formula:

$$2(2+2)$$

but since the miracidium only contains two flame cells on either side, an anterior one and a posterior one, this =  $2(1+1)$ . It is clear that each of these has given rise to another so that the system in the cercaria (which is fork-tailed) is more expressively written as

$$2[(1+1)+(1+1)]=8$$

where each figure 1 represents a branch. Fork-tailed cercariae have flame cells in the tail stem and these are placed in parentheses in the formula. A good example of a fork-tailed cercaria is *Cercaria ocellata* from *Lymnaea stagnalis* with a formula of  $2 [(1+1+1) + (1+1+1+1)] = 14$ .

In the Dicrocoeliidae (Fig. 52) the miracidium has but a single flame cell on each

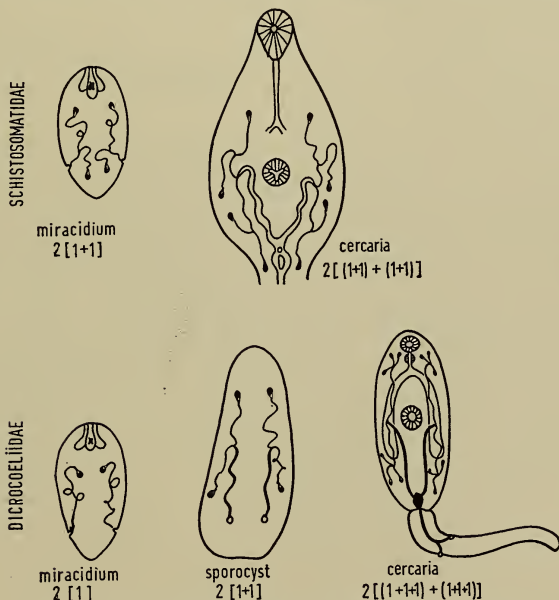


FIG. 52. Flame-cell patterns in larval trematodes (after Dawes, 1946).

side, formula  $2 [(1)]$ , which in the sporocyst gives rise to two on each side  $= 2 [(1 + 1)]$ , each of which gives rise to three flame cells in the cercaria  $= 2 [(1 + 1 + 1) + (1 + 1 + 1)] = 12$ .

A more elaborate system is to use additional letters or other symbols to indicate the branching pattern. Thus, cell No. 1 may give rise to 2 cells,  $1a + 1b$ ; if  $1b$  divided further, the two cells formed would be termed  $1b^1 + 1b^{11}$ ; if  $1b^{11}$  divided again, the cells would be  $1b^{11*} + 1b^{11**}$ , and so on. An example of this formula is that of the

cercaria of *Diplostomum phoxini* (p. 207) which has a formula of  $2[(1a + 1b + 2) + (3 + 4a + 4b^1 + (4b^{11*} + 4b^{11**}))] = 16$ , as shown in Fig. 84.

There are normally two main lateral excretory canals which fuse to form an excretory vesicle opening posteriorly by a pore. In some, this fusion takes place early so that a long excretory vesicle is present, in others it occurs almost at the pore so that the excretory canal is scarcely visible.

*Reproductive system.* Except for two families which are unisexual (the *Schistosomatidae* and the *Didymozoidae*), the digenetic trematodes are hermaphrodite. The reproductive system is invariably extensively developed and although varying in detail, follows essentially the same pattern in most species.

*Male.* Protandry is the general rule in the Digenea. There are usually two testes, vasa efferentia, a vas deferens, seminal vesicle, a ductus ejaculatorius and a cirrus or penis enclosed in a sac or pouch. So-called *prostate* glands may be present. Minor variations from this general plan occur. Spermatogenesis follows the pattern typical of the platyhelminthes, 64 spermatozoa arising in each sperm bundle. The spermatozoa are thread-like with no well-developed head, and spermatogenesis is very sensitive to abnormal environmental conditions such as temperature or pH.

*Female.* The female apparatus contains a single ovary with an oviduct, a receptaculum seminis, a pair of so-called 'vitelline glands' (= vitellaria) with ducts, a chamber where eggs are formed, the ootype, and a collection of gland cells collectively known as Mehlis' gland. The relationship between these structures is shown in Fig. 51. In addition, digenetic trematodes possess a canal, termed *Laurer's canal*, which leads from the oviduct to the dorsal surface of the body and is homologous with the vagina of Monogenea and Cestoda. Many, and possibly all trematodes, possess a muscular chamber or ovicapt (= oocapt)—an enlarged portion of the oviduct where it joins the ovary. This probably controls the release of ova and spaces out their descent down the uterus.

*Fertilisation.* Self-fertilisation or cross-fertilisation may take place, the former probably being the rule. When copulation occurs, the cirrus of one fluke is thrust into the genital opening of the other. When this occurs, it is clear from Fig. 51 that spermatozoa must travel the length of the uterus, possibly against a stream of passing eggs. It is difficult to believe that such an arrangement would be efficient in practice, and it is more likely that spermatozoa are passed down to the receptaculum before egg-formation commences, to be subsequently released for fertilisation as required. Against this latter view, it may be pointed out that the testes continue to produce spermatozoa throughout the life of the fluke. Several instances have been observed of a cirrus being inserted into the

opening of Laurer's canal and fertilisation may take place in this way in some species. The anatomical similarities between Laurer's canal and the vagina in pseudophyllidean cestodes (Fig. 97) is so striking that it is difficult to believe that they both do not serve the same function. In cestodes, of course, the vagina is used exclusively for passing spermatozoa to the ootype.

**Egg formation.** Ova are released periodically from the ovary. At the same time a number

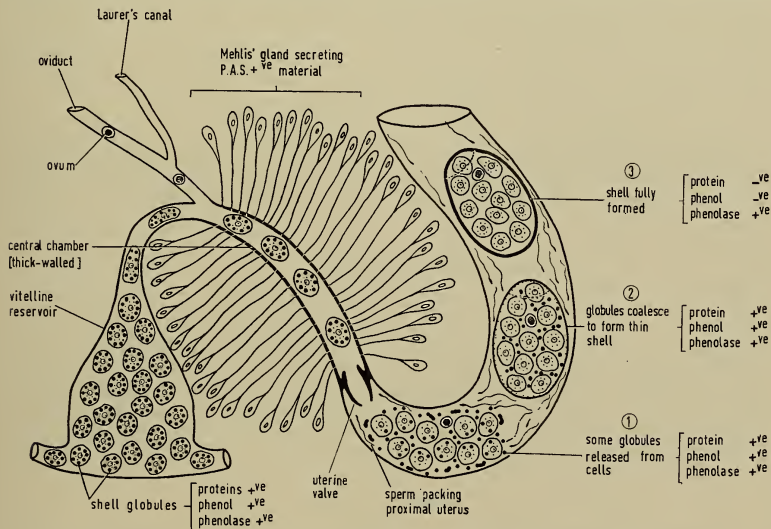


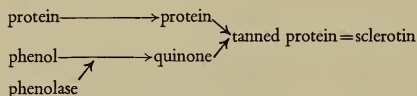
FIG. 53. Mechanism of egg-shell formation in a digenetic trematode; based on *Fasciola hepatica* (from Smyth and Clegg, 1959).

of 'vitelline' cells are released from the vitellaria and spermatozoa from the receptaculum seminis (Fig. 53). Fertilisation occurs in or near the ootype region; only a single spermatozoon taking part in the process; polyspermy does not occur. In species lacking a receptaculum seminis (e.g. *Fasciola*) spermatozoa may be stored in the proximal part of the uterus, sometimes termed the *receptaculum seminis uterinum*. The ootype is surrounded by a number of unicellular glands collectively known as Mehli's gland. It was formerly thought that this gland gave rise to the egg-shell and hence it is often misnamed the 'shell gland'.

The function of this gland remains obscure. In both trematodes and cestodes (see p. 241) its secretion gives a strong histochemical reaction with the periodic acid Schiff (P.A.S.) reaction indicating it to be of the nature of a mucopolysaccharide. Its secretion does not appear to be identifiable with any of the P.A.S.-positive substances known in vertebrates. It is possible that it may be secreting a hormone which plays some part in the synchronisation of the reproductive process, although numerous other suggestions as to its function have been put forward.

It is now known that as in Monogenea and pseudophyllidean cestodes, the outer capsule (= shell) is formed from globules released from the vitelline cells. In the region of the ootype these globules, which pass through the walls of the cells, run together to form a shell which is moulded into shape by the wall of the ootype. How the operculum is formed remains a mystery. When first laid, the shell is soft, but on passing up the uterus, due to the chemical changes outlined below, the egg hardens.

*Chemistry of egg-shell formation.* The egg shell in the majority of trematodes is made up of *sclerotin*, a highly resistant protein widespread in the animal kingdom. Sclerotin is a tanned protein, that is, a protein stabilised by cross-links, rather in the fashion that leather is 'tanned'. The tanning is accomplished by means of a quinone derived enzymatically from a polyphenol in the presence of oxygen. The reaction takes place as follows:



The vitellaria in the majority of species examined have been found to give strong histochemical reactions for proteins, phenols and phenolase, and since many of these reactions give brightly coloured end-products, it is possible to stain the vitellaria and the eggs selectively by histochemical means (Smyth, 1954; Johri and Smyth, 1956). Although all trematodes so far investigated give a positive reaction for phenols, a few have been found to give a negative result for phenolase, suggesting that an alternative system may be involved in some instances (Smyth and Clegg, 1959).

### 13.2 Trematode Life Cycles

Digenetic trematodes have complex life cycles, with rare exceptions, always involving a mollusc host. Six larval stages may occur in a cycle—a miracidium, sporocyst, redia, cercaria, mesocercaria (rare) and metacercariae—but the majority have four or five.

#### 13.21 Hatching

Eggs reach the outside world via the three main body-waste materials—sputum, faeces or urine. In some cases, the eggs develop embryos while still in the body, but the majority remain undeveloped until suitable conditions for embryonation are reached in the outside world. Water or moisture are normally required for further development and eggs which fail to reach an aqueous environment desiccate rapidly. Unlike those



of nematodes and some cestodes, trematode eggs cannot withstand desiccation. A high oxygen tension and a suitable temperature are necessary for embryonation.

The hatching of eggs containing mature miracidia is controlled by a number of factors, the most important being light, temperature and salinity (Standen, 1951).

**Temperature.** Fig. 54 shows that at 28°C. the eggs of *Schistosoma mansoni* hatch rapidly, but high (37°C.) or low (4°C.) temperatures almost completely inhibit the process, although hatching is restored after eggs at these temperatures are returned to 28°C.

**Light.** Light plays a major role in stimulating the hatching processes in operculate eggs. It is believed that on exposure to light a 'hatching substance' (enzyme?) is released which attacks the operculum-bonding

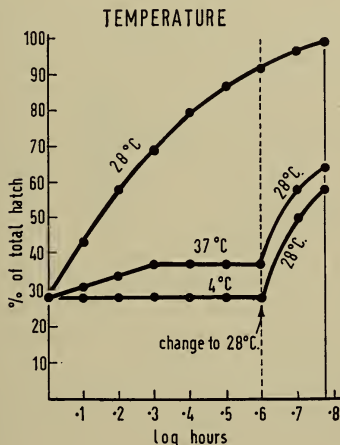


FIG. 54. Effect of temperature on hatching of eggs of *Schistosoma mansoni* (after Standen, 1951).

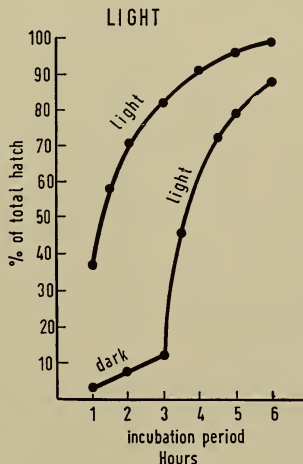


FIG. 55. Effect of light on hatching of eggs of *Schistosoma mansoni* (after Standen, 1951).

material from the inside (Rowan, 1956, 1957). In most trematodes, a small amount of hatching occurs in eggs kept in total darkness, a result probably due to the inevitable exposure of eggs to some light during the screening and concentration processes, but in general it is markedly inhibited (Fig. 55). Hatching takes place most readily in bright sunlight, although a strong electric lamp usually acts as sufficient stimulant; the optimum wave length has not been determined.

**Salinity.** It is clearly to the advantage of the organism that embryonated eggs should not hatch prematurely while still within the host. The inhibitory effect of darkness and high body temperature have already been noted, but with eggs of certain species, osmotic pressure has an even more marked inhibitory effect, within quite narrow limits, and this effect plays a major role in the prevention of premature hatching. Thus hatching of the eggs of *Schistosoma mansoni* is almost completely inhibited by 0.6 per cent

NaCl (Fig. 56) and extensive hatching does not occur until a dilution of 0.1 per cent is reached. Hence eggs in blood, gut contents or urine will only hatch on reaching water. The mechanism of this inhibition is not known.

*Digestive enzymes.* Some eggs, for example those of *Dicrocoelium dendriticum* (p. 166), only hatch on ingestion by a certain species of snail, and it may be presumed that in these cases the snails' digestive enzymes attack the operculum seal.

### 13.22 Larval Forms

*Miracidium.* A typical miracidium is essentially a swimming sac-like larva. Each carries a number of germinal cells from which will arise subsequent generations of organisms.

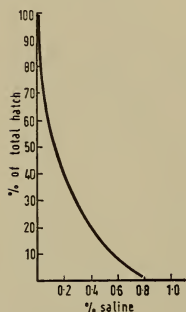


FIG. 56. Effect of osmotic pressure on hatching of eggs of *Schistosoma mansoni* (after Standen, 1951).

In some species (e.g. *Parorchis*) the next generation is formed before the molluscan host has been penetrated, but this is unusual. Gland cells are prominent for assisting penetration into the molluscan host. Miracidia contain only a rudimentary gut, and as they do not feed must rely for energy entirely on endogenous food reserves. These rarely last longer than twenty-four hours, which limits their search for a snail host to this period, after which time they die. There is no evidence that a miracidium finds its host by chemotaxis; it apparently does so by chance. It bores its way into the soft snail tissues, aided by its apical papilla and proteolytic secretions. When it reaches a favourable site, it loses its cilia, and elongates to become an irregular-shaped body termed a *sporocyst*.

*Sporocysts and Rediae.* Sporocysts are essentially germinal sacs containing germinal cells which have descended from the original ovum from which the miracidium developed.

Within the sporocyst, the germinal cells multiply and form new germinal masses. These may either (a) produce daughter sporocysts like the parent sporocyst or (b) produce *rediae*, which are organisms with a rhabdocoele-like intestine, bearing a pharynx and a birth pore. Both of these generations produce embryos which develop into the final generation of organisms termed *cercariae*. If sporocysts give rise to daughter sporocysts, the latter give rise directly to cercariae and rediae are not formed. If sporocysts give rise to rediae before producing cercariae (Fig. 57), these may produce a second or even a third generation of rediae before producing cercariae. The reproductive power of these generations is enormous, and a single egg, by the multiplicative processes of the sporocysts and rediae may ultimately give rise to up to a million cercariae. This is a reproductive phenomenon unsurpassed among the Metazoa.

*Cercariae*. These are essentially young flukes which develop from the germ cells in rediae and sporocysts. During their development, propagatory cells, derived from the original germ cell, give rise to the anlagen of the reproductive system of the adult fluke. Most cercariae bear many of the features of the adult fluke. Most are furnished with a tail, mouth, gut, suckers, flame cells and well-developed histolytic and cystogenous glands. The former secrete proteolytic enzymes for assisting penetration into the second intermediate host. The nature of these enzymes has not been very fully investigated except in a very few forms. In *Schistosoma mansoni*, hyaluronidase and a collagenase

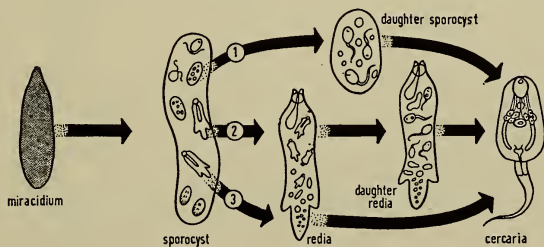


FIG. 57. Pattern of larval development in trematodes (original).

have definitely been detected. Cystogenous glands secrete a cyst wall in those forms which have an encysted stage in their life cycle.

When fully mature, cercariae leave the molluscan host by bursting through the tissue (usually the richly nutrient hepatopancreas) and escaping into the water. Several thousands may be released from a single snail per day. In general, furcocercariae emerge more readily than other cercarial types. The most important factors influencing the release of cercariae appear to be temperature and light, and these effects can be used to control the release for experimental purposes. Thus the cercariae of *Schistosomatium douthitti* normally emerge from their snail hosts in their natural habitats in the evening or in the laboratory after being placed in darkness after exposure to light. The emergence cycle may be reversed by reversing the light cycle (Fig. 80). It is possible that the emergence may be correlated in some way with the habits of the definitive hosts but this question has never been fully investigated. Some unknown factors may also operate, for in some cases the release does not appear to be directly related to the effect of light or temperature. Released cercariae usually show negative geotropism, positive phototropism and thermotropism.

*Classification of cercariae.* The structure of cercariae may provide valuable clues to relationships, often unsuspected, between adults apparently morphologically dissimilar. The number of flame cells, the type of tail (whether single or forked), the presence of a stylet near the oral sucker, are important diagnostic features. Various terms are used

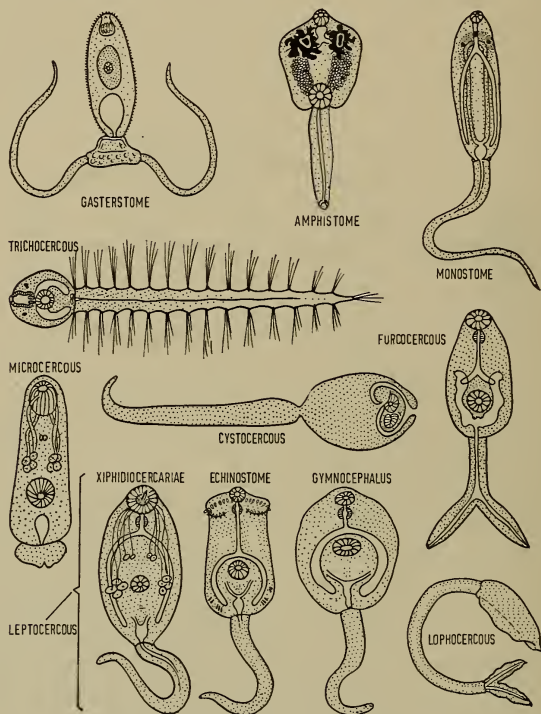


FIG. 58. Various types of cercariae (mainly after Dawes, 1946).

to describe cercariae, and these are based especially on the form of the tail. In addition, the adult characters of certain groups (gasterostomes, monostomes and amphistomes) are sufficiently apparent in their cercariae to make their structure unmistakable, so that these terms are applied to the cercariae also. Thus the cercaria of an amphistome fluke,

which has a large posterior sucker, is easily recognised. The following classification is based on that of Lühe (1909).

*Classification of cercariae* (mollusc host in brackets; based on Lühe, 1909). See Fig. 58.

1. *Gasterostome cercariae*. Two symmetrical tail furcae arising from posterior end of body. Mouth central on ventral surface. Intestine sac-like. Develop in sporocysts. e.g. *Cercaria* of *Bucephalopsis gracilescens* (*Cardium edule*).
2. *Monostome cercariae*. No ventral sucker. Pigmented, two or three eyes. Two excretory canals in body uniting near eyes, one in simple tail. No pharynx. Dense cystogenous glands. Develop in rediae, encyst in open, e.g. *Cercaria monostomi* (*Lymnaea pereger*).
3. *Amphistome cercariae*. Ventral sucker at root of slender tail. Develop in rediae, encyst in open, e.g. *Cercaria* of *Diplodiscus subclavatus* (*Planorbis umbilicatus*).
4. *Rhopalocercous cercariae*. Tail when contracted is wider than body, e.g. *Cercaria isopori* (*Sphaerium corneum*).
5. *Cystocercous cercariae*. Body can be withdrawn into pocket at base of well-developed tail. Tail may be forked or not. Remainder of anatomy very variable. Unnatural group, e.g. *Cercaria macrocerca* (*Sphaerium corneum*).
6. *Gymnocephalous cercariae*. Two almost equal suckers, no stylet, well-developed pharynx, oesophagus and intestine, tail simple. A heterogenous collection, e.g. *Cercaria* of *Fasciola hepatica* (*Lymnaea truncatula*).
7. *Xiphidiocercariae*. Boring stylet with single point. Stylet glands, tail simple. A natural group with the same type of excretory system. Develop in sporocysts, e.g. *Cercaria ornata* (*Planorbis corneus*).
8. *Echinostome cercariae*. Collar of spines around anterior end, tail simple. A natural group, e.g. *Cercaria* of *Echinostoma secundum* (*Littorina littorea*).
9. *Trichocercous cercariae*. Tail with rings of fine bristles, e.g. *Cercaria pectinata* (*Donax vittatus*).
10. *Furcocercous cercariae*. Tail forked distally containing branches of excretory duct. Flame cells in tail stem.
  - (a) Blood fluke cercariae without pharynx, e.g. *Cercaria* of *Schistosoma mansoni* (*Australorbis glabratus*).
  - (b) Strigeid cercariae with pharynx, e.g. *Cercaria* of *Diplostomum phoxini* (*Lymnaea pereger*).
11. *Microcercous cercariae*. Tail vestigial, unnatural group, e.g. *Cercaria micrura* (*Bithynia tentaculata*).



12. *Cercariaea*. Tail absent, unnatural group, e.g. *Cercaria* of *Leucochloridium paradoxum* (*Succinea* sp.).
13. 'Rattenkönig' cercariae. Cercariae tangled by tails to form colony—marine, little known.

Gymnocephalous, echinostome and xiphidiocercariae are sometimes grouped together as 'Leptocercous' cercariae, i.e. cercariae with tail straight, slender and narrower than the body.

*Metacercariae*. Before becoming infective, most cercariae (except those of the Schistosomatidae) must undergo a further maturation phase during which time they are known as *metacercariae*. Released cercariae behave in one of three ways.

(a) *Penetration without encystment*. Cercaria in this group bore into either an intermediate host or a definitive host. The former become localised in relatively deep, soft tissue such as that of the brain or lens. These forms lose their tails and develop into metacercariae but do not encyst (e.g. the cercaria of the strigeid *Diplostomum phoxini*, see p. 207). In the latter group are the cercariae of the Schistosomatidae (p. 188) which mature to adults without the interposition of a metacercaria stage.

(b) *Penetration followed by encystment*. Cercaria in this group bore into an intermediate host and likewise encyst, usually in the skin but they may encyst in any part of the body. Almost any animals, vertebrate or invertebrate, can serve as intermediate hosts to metacercariae—fish, worms, leeches, planaria, etc. The structure of the cyst wall varies considerably with the host. The outer wall is protein, which may or may not be tanned, and an inner lipid membrane is also usually present (e.g. the metacercaria of *Cryptocotyle lingua* in marine fish, see p. 71).

(c) *Direct encystment*. Cercariae in this group attach themselves to foliage or other material, lose their tails and become enclosed in a thin cyst secreted in a matter of seconds by the cystogenous glands (e.g. the metacercaria of *Fasciola hepatica* on foliage, see Fig. 61).

*Maturation of metacercariae*. The time required for metacercariae to become infective after encystment varies from several hours to several months and the degree of morphological development achieved varies correspondingly. The following groups may be distinguished:

(a) metacercariae which show little morphological difference from the cercariae and are infective immediately after encystment (e.g. *Fasciola hepatica*).

(b) metacercariae which may grow in size and perhaps alter in shape but which show no organogeny (e.g. *Diplostomum phoxini*).



(c) metacercariae which show marked organogeny and possess the anlagen of the major reproductive organs (e.g. *Cryptocotyle lingua*).

(d) metacercariae which have developed beyond the stage of organogeny and show advanced spermatogenesis and possibly vitellogenesis. In such forms, mature spermatozoa may be present in the testes and sometimes even in the vas deferens, but ova are never released from the ovary and eggs are never formed (e.g. *Bucephalopsis gracilescens*, see Fig. 60).

(e) metacercariae (often called 'pre-adults') which show progenesis (p. 247) to a varying degree but which produce eggs while still encysted. Some genera (e.g. *Coitoeacum* sp.) produce only a small number of eggs, much less than in the final fish host. Others form what are morphologically true adults although the fertility tends to be poor. Some of these progenetic trematodes exhibit atrophy of the testis and reproduce parthenogenetically (e.g. *Ratzia joyeuxi* under the skin of *Discosaccus*).

A form in snakes, believed to be the normal adult of this species, has normal testes. In still others, e.g. *Paralepoderma brumpti* in tadpoles, the true adult stage is probably reached, for gonadal development and egg-production compares favourably with those of average adult trematodes, 500–1,000 eggs per day. The definitive host for these metacercariae has never been found and experiments have failed to infect animals which on ecological grounds might act as definitive hosts. It is possible, then, that this species exists only in the progenetic condition and the third host has been eliminated from the life cycle. A number of other cases of progenesis is known (Buttner, 1955).

*The germinal cell cycle in trematodes.* The form of larval reproduction in trematodes is a distinctive one and its interpretation has given rise to many controversies, now only of historic interest. The main point of issue has been the origin and development of the germ cells in the various larval stages. All the morphological and cytological evidence now available suggests that the reproductive cells in the sporocysts and rediae can be traced back to the fertilised ovum. These cells, termed *germinal* cells, rather than *germ* cells, remain separate from the somatic cells during the development of the germinal sacs, and undergo no reduction divisions. Therefore the multiplication of such cells is really a polyembryology of the original zygote. The actual tissues of the germinal sacs of miracidia, rediae and sporocysts are split off from the cells of the germinal line. The only cells which undergo meiosis are the spermatozoa and ova in the reproductive cells of the adult.

Alternation of the physical conditions of the environment such as a lowering of the oxygen tension or altered temperature may induce polyembryology in other organisms and it may be that the developmental pattern encountered in the Digenaea is an adaptive response to such environmental changes. As in adult trematodes, the measure of growth achieved is dependent to a large extent on the nutrition available

in the mollusc. The predilection of larval forms for the digestive gland of their molluscan host is suggestive of high nutritional requirements for such stages.

### 13.3 Classification

The classification of trematodes is still in a very uncertain state. The number and arrangement of suckers or other external features has given rise to terms, which

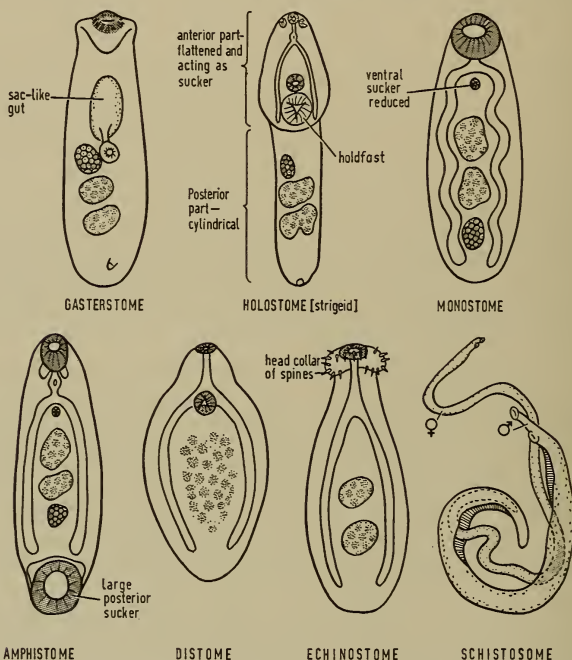


FIG. 59. Diagrammatic representation of the main types of trematodes (partly after Cable, 1949).

although having no precise classificatory significance, are generally widely used and serve as convenient operative terms. The main trematode types are shown in Fig. 59.

*Gasterostome*. Intestine simple and sac-like (e.g. *Bucephalopsis*, see p. 156). Mouth not terminal.

*Monostome*. Generally lacking one sucker, usually the ventral but may be the oral; alternatively one may be greatly reduced. Both suckers may be absent (e.g. *Notocotylus*).

*Amphistome*. Body thick and fleshy with well developed posterior sucker (e.g. *Paramphistomum*, see p. 209).

*Distome*. The mouth is surrounded by an oral sucker and the remaining sucker may be anywhere on the ventral surface except at the posterior end of the body (e.g. *Fasciola*, see p. 158).

*Echinostome*. The oral sucker is surrounded by a very characteristic collar of spines (e.g. *Echinostoma*).

*Holostome* (= Strigeid). Body divided into anterior and posterior regions, the former containing an additional adhesive organ, the holdfast or tribocytic organ (e.g. *Diplostomum*, see p. 203).

*Schistosome*. Elongate dioecious worm-like forms; parasitic in the blood stream (e.g. *Schistosoma*, see p. 185).

A precise classification should take into account the characteristics of the miracidium and cercaria as well as the adult. The development of the excretory system is of fundamental importance in determining relationships, and forms the basis of most systems of classification. The problem has recently been reviewed by La Rue (1957). In the absence of an agreed classification, the group is considered here merely under certain families. Only those which are common, or are of particular interest or importance, are discussed. The order of families given is one of convenience and does not indicate relationship.

1. *Bucephalidae* (p. 156) Mouth near middle of body; sac-like gut; parasites of fishes (e.g. *Bucephalopsis gracilescens*, in angler fish).
2. *Fasciolidae* (p. 158) Large flattened leaf-like forms; much branched intestine; parasites of mammals (e.g. *Fasciola hepatica* in sheep).
3. *Opisthorchiidae* (p. 163) Small to medium transparent forms; parasitic in liver of mammals (e.g. *Clonorchis sinensis* in man).
4. *Dicrocoeliidae* (p. 165) Small transparent forms; vitellaria restricted to behind ventral suckers (e.g. *Dicrocoelium dendriticum* in sheep).
5. *Plagiorchiidae* (p. 171) Small, oval, flattened forms; simple intestine; mainly parasites of amphibia and birds, occasionally mammals (e.g. *Haematoloechus variegatus* in frog).
6. *Echinostomatidae* (p. 173) Echinostomes. Elongate forms with a reniform (kidney-

- shaped) collar surrounding the oral sucker and bearing spines (e.g. *Parorchis acanthus* in birds; but see p. 175).
7. *Heterophyidae* (p. 177) Small forms usually not more than 2 mm long and wider posteriorly than anteriorly. Ventral sucker usually central and may be absent (e.g. *Cryptocotyle lingua* in birds).
  8. *Troglotrematidae* (p. 181) Large or medium-sized trematodes with suckers frequently poorly developed and the ventral sucker sometimes absent; cirrus sac usually absent; parasites of birds and carnivorous mammals, usually in pairs in cysts in various parts of the body (e.g. *Paragonimus westermani* in man).
  9. *Strigeidae* (p. 203) Strigeids or holostomes. Body divided characteristically into an anterior flattened or cup-shaped portion, bearing an additional adhesive organ provided with histolytic glands (e.g. *Alaria alata* in dog, fox).
  10. *Diplostomatidae* (p. 203) Similar to the Strigeidae, but anterior region of body more flattened and having two lateral tentacle-like processes bearing gland openings (e.g. *Diplostomum phoxini* in birds).
  11. *Schistosomatidae* (p. 184) Schistosomes. Dioecious trematodes parasitic in the blood of various birds and mammals (e.g. *Schistosoma mansoni* in man and other mammals).
  12. *Paramphistomatidae* (p. 209) Amphistomes. Large trematodes, usually thick and circular in transverse section; ventral sucker large and situated at or near the posterior end (e.g. *Paramphistomum cervi* in cattle).

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## CHAPTER XIV

### DIGENEA:

### BUCEPHALIDAE, FASCIOLIDAE, DICROCOELIIDAE

#### 14.1 Family Bucephalidae

The gasterostomes characteristically have the mouth in the centre of the ventral surface. The adults occur in the gut of marine and fresh-water fish. The metacercariae are encysted in the nervous system of smaller fish.

#### Type Example: *Bucephalopsis gracilescens*

definitive host:	<i>Lophius piscatorius</i> (angler fish and others)
location:	pyloric caeca, intestine
miracidium:	free-swimming
molluscan hosts:	<i>Mytilus edulis</i> (mussel) or <i>Cardium edule</i> (cockle)
second intermediate host:	various ganoid fish (especially haddock)

*Morphology.* The morphology of the adult, which occurs in the pyloric caeca and duodenum of *Lophius* is shown in Fig. 60. The unusual features presented by this species, and characteristic of the family, are as follows.

The mouth is ventrally located (not terminal as in other trematodes) and the gut is sac-like, resembling the gut of rhabdocoele turbellarians. There is a large excretory bladder often containing pigment; the probable flame-cell formula of the cercaria is  $2 [(2+2)+(2+2)] = 16$ . The common genital pore is posterior. The reproductive organs characteristically (Fig. 60) have an elongate cirrus with glandular walls.

*Life cycle.* The adults produce large numbers of dark brown eggs with an unusually thick shell. Unlike most trematodes, this shell, although giving phenolic reactions,



does not contain phenolase and is not apparently tanned and hardened by the usual quinone tanning system (p. 144).

Embryonation takes place in sea water. The miracidium which hatches is a grotesque organism with the cilia restricted to comb plates and protruding bars.

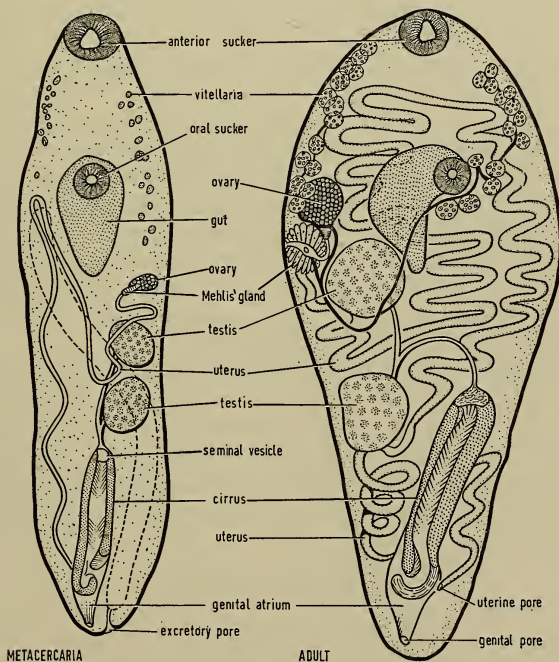


FIG. 60. *Bucephalopsis gracilescens*. Progenetic metacercaria with mature spermatozoa in the testes; from brain of *Gadus merlangi*. Adult from the gut of angler fish *Lophius piscatorius* (from an original drawing by Dr. D. I. Howie).

Miracidia are drawn into the molluscan host via the inhalent siphon and develop into sporocysts which branch and ramify through the digestive gland and even reach the gills and gonads, sometimes causing castration. Some of the branches are probably nutritive and serve to supply food materials to the germinal tubes which make up the remainder of the sporocysts. The cercariae become attached to the fin rays of members

of the family Gadidae, especially haddock, being helped in this process by their forked prehensile tails. Penetration is effected by means of histolytic glands, and the tail is lost. Cercariae show a marked predilection for the nervous system, especially the posterior cranial nerves on which they settle and develop into metacercariae. These become enclosed in a soft-walled cyst, probably of host and parasite origin. The nutriment available in the brain cavity is probably low in protein but is sufficient for an advanced stage of morphological development to be achieved, and partial progenesis (p. 247) to occur. In the most 'advanced' metacercariae, the major genitalia are differentiated (Fig. 60), *spermatozoa are present in the testes and seminal vesicles*, and the vitellaria contain shell precursors which give reactions for phenols. The nutritional level is thus sufficient to satisfy the demands of the gametogenesis level of organisation, but not sufficient for egg-production. On ingestion by *Lophius*, the metacercariae are carried to the stomach, duodenum or pyloric caeca, where the nutritive level is higher than that in the brain, and sufficient to overcome the nutritive barrier apparently separating it from maturation. At 18°C., maturation to the egg-producing stage requires about twenty-one days. The Q<sub>10</sub> for the maturation is about 3, and the upper limiting temperature above which normal development will not occur is about 22°C.

*General Account.* *B. gracilescens* is common in European waters but does not occur around the United States due to the absence of the molluscan host. Many other species are well known throughout the world but the taxonomy is in a somewhat confused state (Hopkins, 1954, 1956). Some species with larval stages in oysters are of economic importance. The life cycles of bucephalids are very imperfectly known (Woodhead, 1929, 1930), being based mainly on morphological resemblances between various stages, and await experimental verification.

## 14.2 Family Fasciolidae

### 14.21 Genus *Fasciola*

Large distomes with broad, flat, muscular bodies. Most organs complicated by branching. Includes several species of economic importance.

#### Type Example: *Fasciola hepatica*

natural definitive hosts: sheep, cattle and others

suitable laboratory host: rabbit

habitat: bile ducts

miracidium: free-swimming

intermediate host: *Lymnaea truncatula* (Europe)

metacercariae: encysted on vegetation

*Occurrence.* Occurs most commonly in sheep, goats and cattle, although rabbits are readily infected experimentally. The usual site of infection is the liver but in aberrant hosts (such as man) other sites, such as the lung, may be involved.

*Morphology.* This is too well known and described in elementary texts to warrant detailed description here. The cuticle is well armed with backwardly directed spines, which together with the suckers serve as an effective mechanism to maintain the position of the parasite in the bile duct. Mehlis' gland is especially well developed as is Laurer's canal. The egg shell is the usual quinone-tanned protein formed by the mechanism described on p. 143.

*Life cycle.* The life cycle follows the typical trematode pattern (Fig. 61). The eggs embryonate in water in about seventeen days at 22°C., and on exposure to light hatch, probably due to the release of the 'hatching enzyme' (p. 145) which attacks the opercular cement. The life of a hatched miracidium is only about twentyfour hours. The most commonly infected snail in Europe is *Lymnaea* (*Galba*) *truncatula*, but a large number of other hosts has been incriminated (Table 19).

TABLE 19

INTERMEDIATE HOSTS OF *FASCIOLA HEPATICA* IN DIFFERENT COUNTRIES

<i>Species of snail</i>	<i>Country</i>	<i>Species of snail</i>	<i>Country</i>
<i>Lymnaea truncatula</i>	Europe	<i>Galba bulimoides</i>	U.S.A.
<i>Lymnaea palustris</i>	Europe	<i>Galba bulimoides techella</i>	U.S.A.
<i>Lymnaea pereger</i>	Europe	<i>Galba ferruginea</i>	U.S.A.
<i>Lymnaea glabra</i>	Europe	<i>Galba cubensis</i>	U.S.A.
<i>Lymnaea stagnalis</i>	Europe	<i>Fossaria modicella</i>	U.S.A.
<i>Lymnaea philippinensis</i>	Philippines	<i>Lymnaea traskii</i>	U.S.A.
<i>Lymnaea swinhoei</i>	Philippines	<i>Pseudosuccinea columella</i>	U.S.A.
<i>Amphipepla cumingiana</i>	Philippines	<i>Lymnaea acuminata</i>	India
<i>Lymnaea launcestonensis</i>	Australia	<i>Lymnaea viatrix</i>	Argentina
<i>Simulimnaea tomentosa</i>	New Zealand	<i>Lymnaea pervia</i>	Japan
<i>Lymnaea alfredi</i>	New Zealand	<i>Lymnaea japonica</i>	Japan

Kendall (1949) has shown that the development of *F. hepatica* in species other than *L. truncatula* is limited by their relative resistance to infection and by the pathogenicity of the parasite. *L. truncatula* may be infected at any age or size, but all the other British species (Table 19) are susceptible only during the first few days of hatching, so that the chances of their natural infection are small. In the field only about 5-7 per cent of *L. truncatula* are infected, which suggests that other species do not play a significant part as vectors, at least in Britain.

*L. truncatula* is a non-operculate pulmonate snail. It is essentially amphibious in habit and spends more time out of water than in it; it is an inhabitant of temporary ponds and muddy places which may become dry for part of the year. It is thus admirably suited to transmit a parasite to grazing cattle. It has been shown experimentally that all stages, eggs, young and adults, can withstand desiccation for considerable periods, the adults for periods of a year or more, provided they are favourably covered with mud. The contained

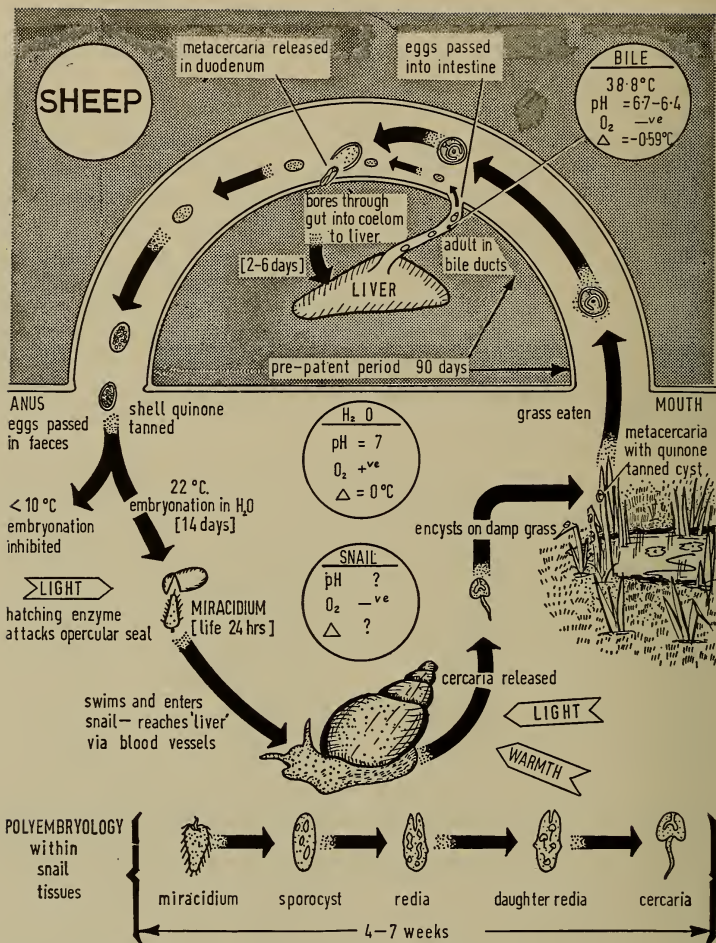


FIG. 61. *Fasciola hepatica*—life cycle and some of the physiological factors concerned in it (original).

sporocysts or rediae are thus directly protected against detrimental environmental conditions. *L. truncatula* breeds with great rapidity, maturing only twenty-one days after hatching, at average temperatures (16–20°C.) It has been estimated that a single snail can potentially produce 160,000 snails within twelve weeks.

*Development within the molluscan host.* The delicate miracidium penetrates a snail assisted by the secretion of its histolytic glands and reaches the digestive gland via the lymph channels. Sporocyst and redia generations develop. The rate and extent of development depend mainly on two factors, (a) the available food reserves in the gland, and (b) the size of the infection. In starved or hibernating snails, development is greatly retarded (Table 20), but recovers rapidly when the snail resumes feeding. In heavy

TABLE 20

COMPARISON OF THE NUMBER OF REDIAE AND FULLY FORMED  
CERCARIAE IN FED AND STARVED *L. TRUNCATULA*  
(data from Kendall, 1949)

<i>Well-fed snails</i>			<i>Starved snails</i>		
<i>Shell length</i> (cm)	<i>No.</i> <i>rediae</i>	<i>No.</i> <i>cercariae</i>	<i>Shell length</i> (cm)	<i>No.</i> <i>rediae</i>	<i>No.</i> <i>cercariae</i>
0.96	151	1,353	0.62	133	200
0.82	296	1,308	0.74	215	218
0.92	141	1,078	0.72	169	374
1.02	133	1,299	0.67	215	134
0.82	152	952	0.74	119	391
0.93	156	1,390	0.61	159	14
0.93	136	1,160	0.68	196	9
1.09	136	1,593	0.62	30	131
1.22	344	2,275	0.63	57	292
1.08	200	2,018	0.62	82	480

infections, even in well-fed snails, there is competition for available nutriment and the maximum number of rediae allowing rapid development appears to be about the forty level; beyond this number development is considerably retarded. Since a single miracidium infection gives rise to about forty rediae, it would appear that the economy of the snail is adapted to infections at approximately this level. Laboratory infections are normally heavier (Table 20).

*Cercaria production.* A single miracidium can ultimately give rise to about 600 cercariae. Emergence commences some five to six weeks after the initial infection at average temperatures. The factors which stimulate cercaria production in *Fasciola* are not understood. Temperature has a threshold effect only. Below 9°C. emergence is entirely inhibited, and as the snails cannot survive for long at 26°C., this is an upper limiting temperature for cercarial emergence also. Between these limits emergence is apparently unaffected by temperature. Factors such as light or darkness, pH, O<sub>2</sub> tension, have been shown



experimentally to have no effect, and yet in the laboratory the act of transferring infected snails into fresh water invariably stimulates cercaria production (Table 21).

TABLE 21

THE EFFECT OF VARIOUS CONDITIONS ON THE EMERGENCE  
OF CERCARIAE OF *F. HEPATICA*

Fifty snails were used in each group (data from Kendall and McCullough, 1951)

Conditions	Number from which cercariae emerged
Snails undisturbed . . . . .	1
Snails returned to same water after mechanical disturbance . . . . .	2
Snails placed in water previously occupied by other snails . . . . .	6
Snails placed in fresh pond water . . . . .	27

This stimulation is associated in some way with the activity of the host, but its basis is at present unknown. Rainfall has a similar stimulating effect in the field.

The cercariae are gymnocephalous in type (p. 149) and have well developed cystogenous glands. On emergence from the snail, a cercaria anchors itself by means of its oral sucker to a suitable substrate, such as grass, loses its tail and secretes a cyst by means of its cystogenous glands.

Like the egg shell, the cyst wall is a quinone-tanned protein and darkens and hardens rapidly on exposure to air. In snails maintained in the laboratory, encystment takes place at or near the air/water surface and if the vessel is lined with cellophane, encysted metacercariae can be conveniently collected.

The maximum survival time of encysted metacercariae is not known, but at average temperatures (Gt. Britain) it is about two weeks on dry grass but probably several months on moist grass or hay.

On ingestion by the definitive host, the metacercariae hatch in the duodenum due to the action of unknown factors; these are probably proteinases and lipases which remove both the protein and lipid layers respectively. Released metacercariae immediately penetrate the duodenal wall, assisted by their histolytic glands, and reach the coelom, where they may be found some two hours after infection. After two to six days, the liver capsule is penetrated and the juvenile flukes live in the liver parenchyma, eventually reaching the bile duct in which they become mature some seven weeks after the initial infection.

*Nutrition.* The nature of the food of *Fasciola* has long been in dispute, the main point of disagreement being whether or not the organism feeds on blood. Although histochemical evidence has been negative, the recent use of radioactive tracers (p. 214) has shown that blood is ingested by the organism (Jennings *et al.*, 1954, 1955). Nothing is known regarding the process of digestion.



*Other Species:* *F. gigantea*. A trematode resembling *F. hepatica* but larger in size (2.5—7.5 × 1.2 cm). It is a common liver fluke of domestic and wild ruminants in many parts of Africa and the orient. The life cycle resembles that of *F. hepatica*. The intermediate hosts are: S. Africa, *Lymanaea natalensis* and *Physopsis africana*; Hawaii, *Fossaria ollula*; India, *Lymanaea acuminata*.

#### 14.22 Genus *Fascioloides*

*Fascioloides magna*. Occurs in the liver (and occasionally lung) of deer in N. America, but can also infect horses, sheep and cattle.

In cattle, the flukes become entirely encapsulated before reaching maturity so that eggs are never released. The pathogenicity of these hosts is low. In deer, the capsule is developed later and eggs may escape. In sheep, the liver may become severely affected by migration and the presence of two or three of these large flukes may prove fatal. The parasites contain a characteristic black pigment related to melanin, presumably derived from haemoglobin, but whose composition is unknown. The life cycle is similar to *F. hepatica*. The intermediate hosts are: *Fossaria parva*, *F. modicella*, *F. modicella rustica*, *Galba bulimoides techella*, *Pseudosuccinea columella*, *Stagnicola palustris nuttalliana*.

*Fasciolopsis buski*. A duodenal parasite occurring in pigs and man. Its general morphology is similar to that of *Fasciola* but it lacks the thickened anterior cone and it has unbranched intestinal caeca. It is widespread in Asia, occurring mostly in China, but has been found in other parts of the world. In some areas in China, it constitutes an important health problem and 100 per cent of local populations may be affected.

*Life cycle*. Resembles *F. hepatica* with members of the family Planorbidae, principally *Segmentina hemisphaerula* and *Hippeutis contori*, as intermediate hosts. These snails feed on plants such as the water calthrop, *Trapa natans* and *T. bicornis*, and the water chestnut *Eliocharis tuberosa*—much cultivated for food and fertilised with human night soil. The nuts or bulbs of these plants are eaten raw, the nuts of the calthrops being peeled by the teeth so that heavy, and sometimes lethal infections may occur.

*Nutrition*. Although an intestinal parasite *F. buski* does not feed only on intestinal contents, but also on blood and tissue exudants from irritation sites.

#### 14.3 Family Opisthorchiidae

All members of this family are parasites of fish-eating mammals particularly in Asia and Europe. They are unusually transparent in whole-mount preparations and their general anatomy is readily seen (Fig. 62). The eggs are embryonated when laid and

contain miracidia. Hatching only occurs when eggs are ingested by a suitable snail (p. 146).

*Clonorchis sinensis*. An important parasite of man and fish-eating mammals of the Far East, mainly Japan, Korea, China, Formosa and Indo-China. The main mammalian hosts are cats and dogs. Localised heavy infections are common in Japan.

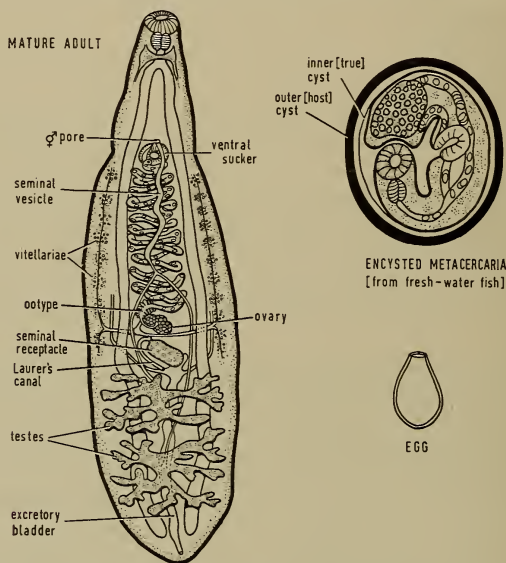


FIG. 62. *Clonorchis sinensis*—morphology of adult, metacercaria and egg (after Brown, 1950; and Komiya and Tajimi, 1940).

*Life cycle* (Fig. 63). The adults live in the bile ducts, often occurring in vast quantities, numbering several thousands. Like *Fasciola*, they feed on blood, but nothing is known of their nutritional requirements. The eggs are shaped like a vase with the operculum fitting into a flared rim (Fig. 62). The intermediate hosts are the snails *Parafossarulus manchouricus*, *Bulinus fuchsianus* and *Alocinma longicornis*. Sporocyst and redia generations occur. The cercariae are exceptional in possessing eye-spots; penetration and cystogenous glands are also well developed. The cercariae penetrate the skin of fishes of the family Cyprinidae, in whose muscles they eventually settle, lose their tails and encyst.

Infection is by eating uncooked, commonly smoked, fish and the excysted metacercariae make their way to the liver, not via the coelom as in *Fasciola*, but by direct migration up the bile duct. Cats and dogs act as reservoir hosts.

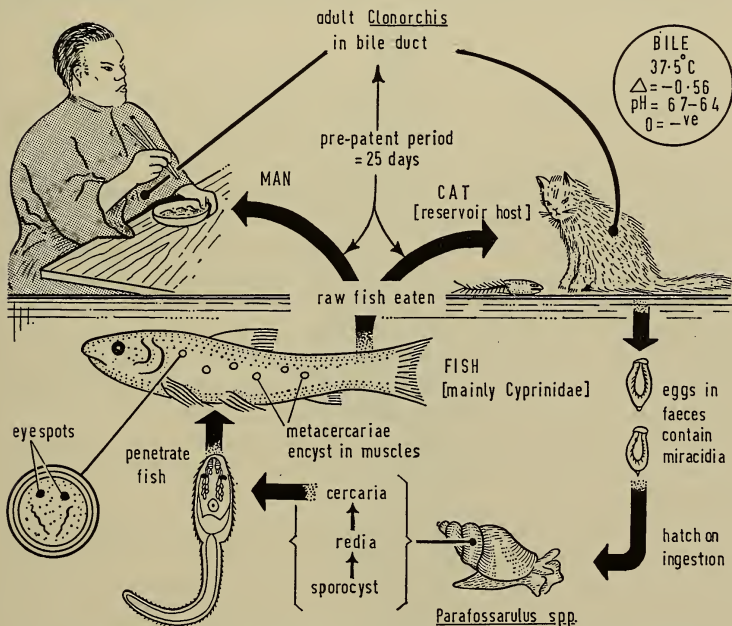


FIG. 63. *Clonorchis sinensis*—life cycle and some physiological factors relating to it (original).

*Opisthorchis felineus*. A species resembling *C. sinensis*, but differing from it in possessing rounded instead of branched testes. It occurs in cats, dogs and other carnivores, from Europe to Japan, occasionally becoming a human parasite. The intermediate molluscan host is *Bulinus tentaculatus*, and the fish hosts, tench, bream, carp, and barbel.

#### 14.4 Family Dicrocoeliidae

The members of this family are delicate, elongate, flattened, rather translucent distomes with suckers not far apart in the anterior region of the body. Flame-cell formula of adult:

$$2 [(2+2+2)+(2+2+2)] = 24.$$

The cercariae are xiphiid cercariae. Although the best-known species occur in mammals, species also occur in amphibians, reptiles and birds.

**Type Example:** *Dicrocoelium dendriticum* (=the lancet fluke)

definitive hosts:	sheep, cattle, deer, woodchuck, rabbit and other mammals
location:	bile ducts
miracidium:	freed only in molluscan hosts
first intermediate host:	<i>Cionella lubrica</i> (U.S.A.). <i>Planorbis marginatus</i> , <i>P. complanatus</i> , <i>Arion</i> and <i>Limax</i> sp. (Europe)
second intermediate host:	the ant, <i>Formica fusca</i> (U.S.A.)

**Occurrence.** The lancet fluke is restricted in its distribution largely to Europe and Asia and only sparsely distributed in Africa, the New World and Australasia. Its appearance in the New World is comparatively recent, being first reported in Canada in 1931 and in the United States in 1940. In the British Isles, it is particularly prevalent in Scotland. Its distribution closely follows that of certain terrestrial snails.

**Morphology.** The internal organs are widely spaced, and this, together with the transparency, makes the morphology especially easy to study in whole mounts. The anatomy, as shown in Fig. 64, is simple and will not be described here. The eggs are operculate and the egg shell probably a quinone-tanned protein.

**Life cycle** (Fig. 65). Some major problems in the life cycle and mode of transmission remained unsolved until the meticulous work of Krull and Mapes (1951-1956) led to their elucidation.

It was formerly thought that mammals became infected by ingesting 'slimeballs', peculiar slimy secretions produced by terrestrial snails and known to contain cercariae. It was suspected by some that a second intermediate host was necessary to complete the life cycle. Krull and Mapes in 1952 showed that a second intermediate host, an ant, is involved, and infection of the definitive host is brought about only by ingestion of infected ants, and cannot take place by direct ingestion of slimeballs.

The operculate eggs passed in the faeces are embryonated when laid, but do not hatch on exposure to light, as do the majority of trematode eggs. Hatching only takes place when the eggs are ingested by the appropriate molluscan host, so that as in certain other families (e.g. Opisthorchiidae and Plagiorchiidae) a light-released opercular-attacking enzyme is not produced.

**Intra-molluscan development.** The intermediate host in the United States is the snail *Cionella lubrica*, and in some other countries this snail also serves as a host. Other snail hosts involved are: *Helicella candidula*, *H. itala*, *H. ericetorum*, *Zebrina detrita*, *Abida*

*rumentum*, *Ena obscura*, *Euomphalia strigella* and *Theba carthusiana*. Hatching takes place in the snail's gut. The miracidia make their way to a site near the albumen gland, between the liver follicles or along the intestine. Mother sporocysts and daughter sporocysts are produced, the latter giving rise to xiphidiocercariae, long known as *Cercaria*

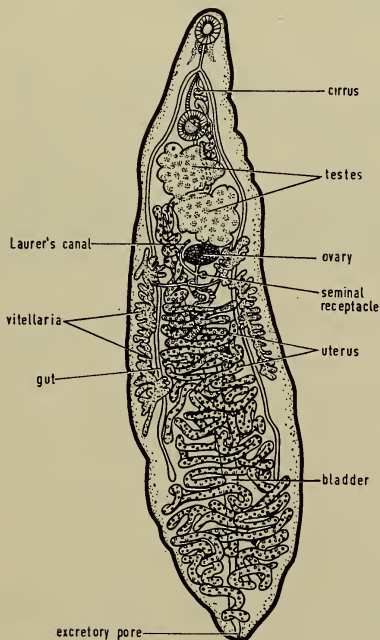


FIG. 64. *Dicrocoelium dendriticum*—morphology of adult (after Neuhaus, 1938).

*vitrina*. Development from miracidium to cercaria takes approximately four to five months at average temperatures, but the rate of development may be influenced by other factors, such as season or age of the snail. Cercariae escape from the snail, and are collected in slime masses known as *slimeballs*, the formation of which is not fully understood.

The cercariae contain massive glands, formerly considered to be penetration glands, but now believed to produce the slime responsible for the formation of these balls. It is likely that the slime produced by the



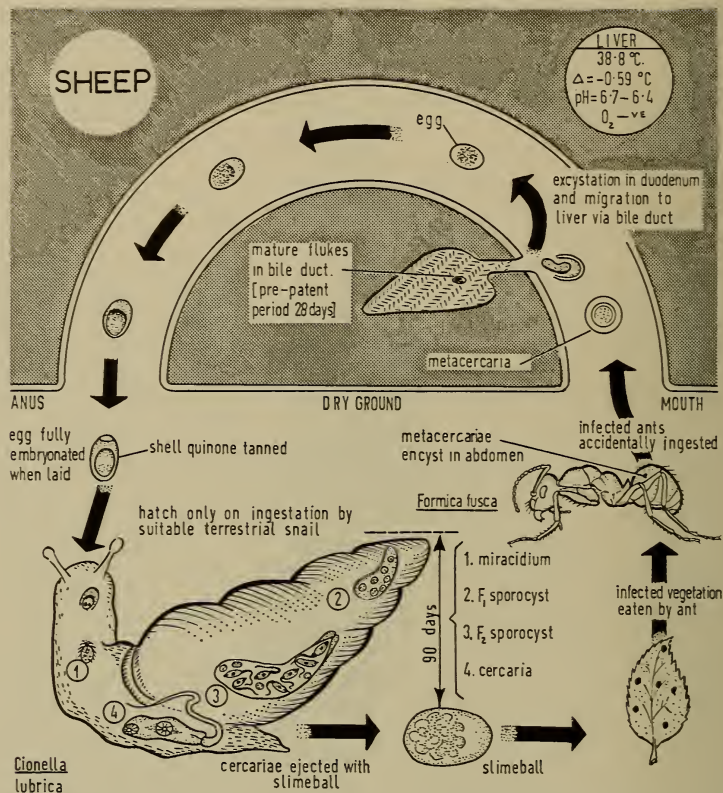


FIG. 65. *Dicrocoelium dendriticum*—life cycle and some physiological factors relating to it (based on the work of Krull and Mapes (original)).

snail itself may contribute, at least partly, to the formation of a slimeball. Slimeballs can be released in at least two ways, either by expulsion of a fully-formed slimeball from the respiratory pore, or by a method involving the use of the surface slime of the snail in which the cercariae, resulting from the sudden exodus from the snail (perhaps induced by a drop in temperature) are incorporated. The balls are sticky in constituency and adhere to vegetation and other debris. Usually only one slimeball is produced by a snail, but up to five may be produced; each ball may contain 100-400 cercariae.



In an excessively humid environment, slimeballs liquefy, and the cercariae die. In an arid environment, in which the snails normally live, the surface dries and the balls shrink to some extent, but the cercariae survive for a period of time.

*Development in the second intermediate host.* On ingestion by the ant, *Formica fusca*, in North America and possibly other species in other countries, the cercariae become transformed to metacercariae in the body cavity of the abdomen, presumably after penetrating the intestine of the ant; thirty metacercariae being an average infection, but up to 218 have been reported. Within the body cavity the metacercariae grow, although remaining semi-transparent. Metacercariae may complete their growth and become infective in somewhat more than a month. A typical metacercaria is shown in Fig. 65.

*Natural definitive hosts.* The definitive host becomes infected by swallowing infected ants and the metacercariae are excysted in the duodenum. The released metacercariae make their way up the bile ducts and the flukes reach maturity in the liver. This route to the liver is in contrast to that of *Fasciola hepatica*, the excysted metacercariae of which bore through the alimentary canal and reach the coelom within two hours. The liver is finally reached, in the case of *Fasciola*, by penetration of the liver capsule (Fig. 61).

*Dicrocoelium* is exceptionally non-specific in its choice of hosts and infections from some forty different hosts have been reported. Human infections have been reported, but their authenticity is open to question. In the United States, at least, deer, woodchuck and the cottontail rabbit serve as definitive reservoirs for *Dicrocoelium*, (Krull, 1956). It is worth recording that it was by the study of the feeding habits of the woodchuck that Krull and Mapes indirectly discovered the role of the ant as the second intermediate host.

*Laboratory hosts.* In the laboratory, *Dicrocoelium dendriticum* is most readily maintained in the golden hamster or the rabbit, but the development can also occur in the guinea pig and albino mouse, although the latter two are poor hosts. The albino rat is refractive to infection. The maximum size reached varies enormously with the different hosts species, indicating markedly different growth rates. Approximately maximum sizes are as follows: mouse, 3.5 mm; guinea pig, 7.5 mm; golden hamster, 8.5 mm; rabbit, 13.0 mm.

For experimental purposes, the hamster is the host of choice for not only does the parasite reach a fair size, but in this host its distribution is confined to the main bile ducts at the surface of the liver, and in the gall bladder.

*Physiology.* Little is known regarding the nutrition and general physiology of *D. dendriticum*. Haemoglobin has been detected in its tissue, so that it can be assumed that, as in *Fasciola*, blood actually forms part of the ingested food material.

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## CHAPTER XV

### DIGENEA:

### PLAGIORCHIIDAE, ECHINOSTOMATIDAE, HETEROPHYIDAE, TROGLOTREMATIDAE

#### 15.1 Family Plagiorchiidae

This family contains some species of trematodes readily available for laboratory study. Many are commonly occurring parasites in the amphibian or reptile types normally dissected in zoology courses. A few species infect homoiothermic animals. The best known genera in Europe are: *Haplometra* and *Haematoloechus* in the lungs and *Dolichosacchus* and *Opisthioglyphe* in the intestine of the frog. All species have stylet cercariae (xiphidiocercariae) which encyst in arthropods (usually insects) or occasionally vertebrates.

#### Type Example: *Haplometra cylindricea*

definitive hosts:	<i>Rana</i> spp., <i>Bufo</i> spp.
location:	lungs
molluscan hosts:	<i>Lymnaea ovata</i> , <i>L. stagnalis</i>
second intermediate host:	<i>Ilybius fuliginosus</i> (Coleoptera)

**Morphology.** This is a common species in European frogs. It is unusual in being almost cylindrical in transverse section. Its general anatomy is shown in Fig. 66.

**Life history.** The lung provides an environment with a high oxygen tension; it is also rich in mucus and has an abundant blood supply. The presence of the fluke in the lung apparently does little damage; the epithelium, which is pavement in the uninfected lungs, becomes columnar. Histochemical tests on the contents of the gut show the presence of haemoglobin and breakdown products of haemoglobin, such as porphorin. The fluke is clearly haematophagous, and the body tissues are rich in iron, but this is bound to an organic compound and histochemical tests for ionic iron are negative.

The eggs of *Haplometra* rapidly turn brown on exposure to air and are presumably tanned protein (p. 144). They are embryonated when laid. As in members of the *Opisthorchiidae*, the eggs only hatch on ingestion by suitable snails. The life cycle is not known in detail, but is similar to that of *Haematoloechus* illustrated in Fig. 67. The molluscan hosts are *Lymnaea ovata* and *L. stagnalis* in which sporocyst stages develop. There are no rediae. The cercariae are cystocercous forms which escape and penetrate into the haemocoel of the larva of the beetle *Ilybius fuliginosus*. The haemocoel is a

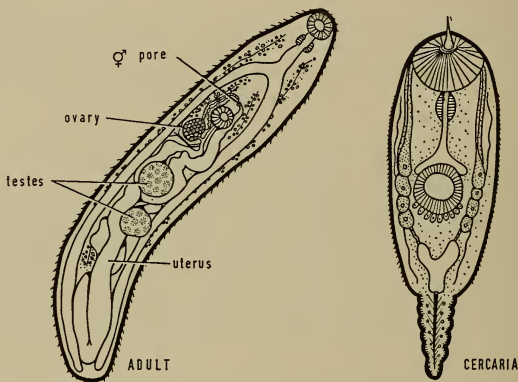


FIG. 66. *Haplometra cylindrica*—adult (from lung of frog) and cercaria (after Dawes, 1946).

nutritive medium, rich in protein and, by diffusion, the encysted metacercariae theoretically could be raised to the organogeny level of nutrition (p. 22), although this phase of the life history has not been studied. This condition actually occurs in the case of *Lecithodendrium chilostomum* (a parasite of bats) whose encysted metacercariae reach a highly advanced state of morphological development in the haemocoel of the larvae of the caddis fly, *Phryganea grandis*. The time required for maturation of *H. cylindrica* in the frog, or its route to the lungs, is unknown.

The following species are also common amphibian parasites:

*Haematoloechus* spp. (Fig. 68). In amphibian lungs. Life cycle shown in Fig. 67. Metacercariae in larval dragonflies.

*Dolichosaccus rastellus* (Fig. 68). An extremely common intestinal plagiorchid in amphibia. Molluscan host *Lymnaea stagnalis*, *L. auricularis* (Europe). Metacercariae in larval Trichoptera and Ephemeroptera.

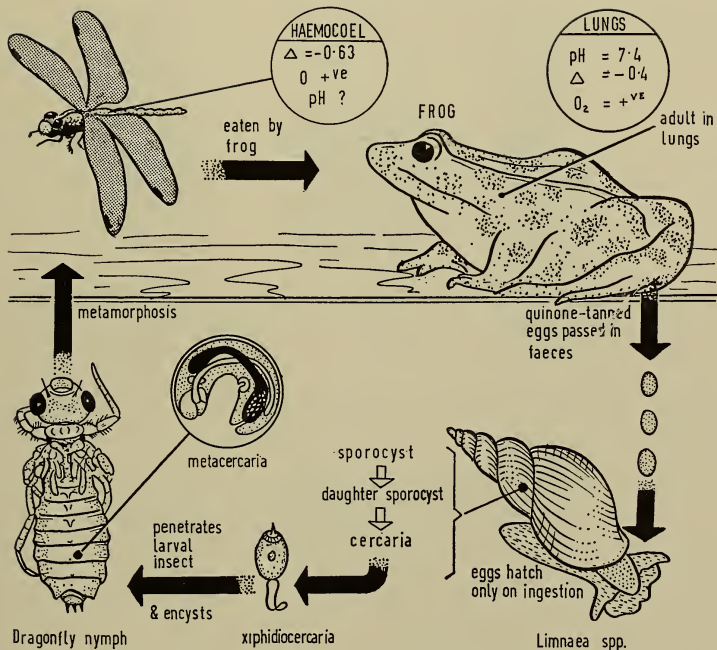


FIG. 67. *Haematoloechus variegatus*—life cycle and some physiological factors relating to it. The eggs are probably embryonated when laid. The details of the cycle are very imperfectly known (original).

*Opisthioglyphe ranae* (Fig. 68). Another common intestinal form in frogs. Molluscan hosts, *L. palustris* and *L. stagnalis* (Europe). Metacercariae in tadpoles and young frogs.

### 15.2 Family Echinostomatidae

Possession of a head collar surrounding the oral sucker, separates the echinostomes morphologically from the remainder of the trematodes. There is no other outstandingly unusual feature. All members of this family are parasites of birds or mammals. Their habitats range along the entire length of the alimentary canal from the duodenum to the caecum and rectum; the bile duct is invaded by some species. The life cycle differs from that of *Fasciola* in but one minor point, the cercariae encyst either within the tissues

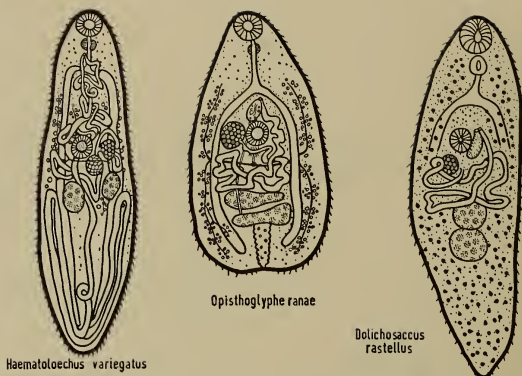


FIG. 68. Three common members of the family Plagiiorchiidae from the frog (after Dawes, 1946).

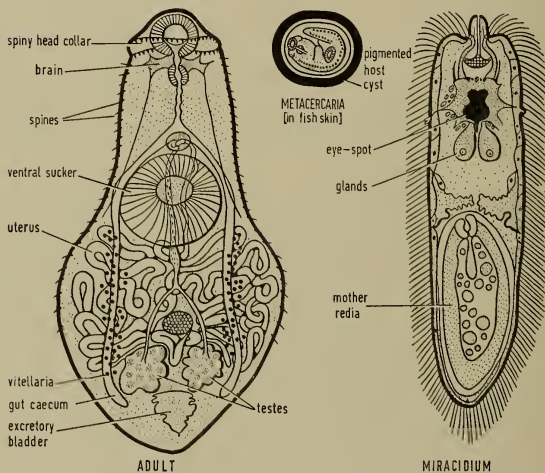


FIG. 69. *Parorchis acanthus*—from rectum or bursa Fabricii of gull. Morphology of adult, miracidium and metacercaria (after Rees, 1939, 1940).



of the same molluscan host in which sporocysts and rediae develop, or penetrate and encyst in other animals such as planarians, molluscs, amphibians or fish. Like the adults, cercariae bear collars of spines.

#### 15.21 Type Example: *Parorchis acanthus*\*

definitive hosts:	herring gull, common gull, tern, flamingo
location:	rectum, bursa Fabricii
molluscan hosts:	<i>Nucella (Purpura) lapillus</i>
transport hosts:	shelled molluscs
distribution:	widespread

**Morphology.** The morphology is shown in Fig. 69. The head collar bears about sixty spines and the cuticle is heavily spined in the region anterior to the ventral sucker becoming sparser behind. Except that the cirrus is spined, the reproductive system presents no unusual features.

**Occurrence.** This is an important species biologically since it is one of the few trematodes in which gametogenesis and larval development have been investigated cytologically in great detail (Rees, 1939, 1940). Infections of up to about twelve flukes can occur in a single host. In Europe, the larval stages occur in the marine snail *Nucella lapillus*, a high proportion of which are commonly infected.

**Life cycle** (Fig. 70). Whatever mechanism retards embryonation in most trematode eggs while within the host, it is lacking here and as the eggs pass up the uterus they increase slightly in size, and the whole process of larval development becomes enormously accelerated. Mature miracidia develop in eggs still *in utero*, and hatching may even occur there. Hatching normally, however, takes place in sea water shortly after being laid. A miracidium, in addition to containing the usual larval features such as eye spots, penetration glands, and a vestigial gut, is almost unique in containing a mother redia with a well developed pharynx, an intestine and germinal balls (Fig. 69). The miracidium penetrates *Nucella* and the mother redia is liberated. The germinal balls contained in the latter form daughter rediae which ultimately reach the digestive gland, finally producing typical echinostome cercariae (Fig. 58).

Released cercariae will encyst on almost any convenient surface, such as snail shells, petri dishes or the walls of the aquarium.

Encystment is accelerated by mechanical stimulation such as stirring or shaking. The process takes place rapidly, the cystogenous material being poured out in a matter of minutes. The nature of the cyst

\*Some authorities (e.g. Yamaguti, 1958) consider this an aberrant echinostome and place it in a separate family, the Philophthalmidae.

wall is unknown, but is probably a tanned protein like that of *Fasciola*. There is no evidence that the cercariae can penetrate into other molluscs, as is sometimes stated, but they can encyst on the surface of exposed soft parts of molluscs such as *Mytilus* or *Cardium*, and such food probably acts as the means of infecting gulls in the normal cycle. Metacercariae once encysted are immediately infective and a larval maturation phase is not necessary (Stunkard and Cable, 1932).

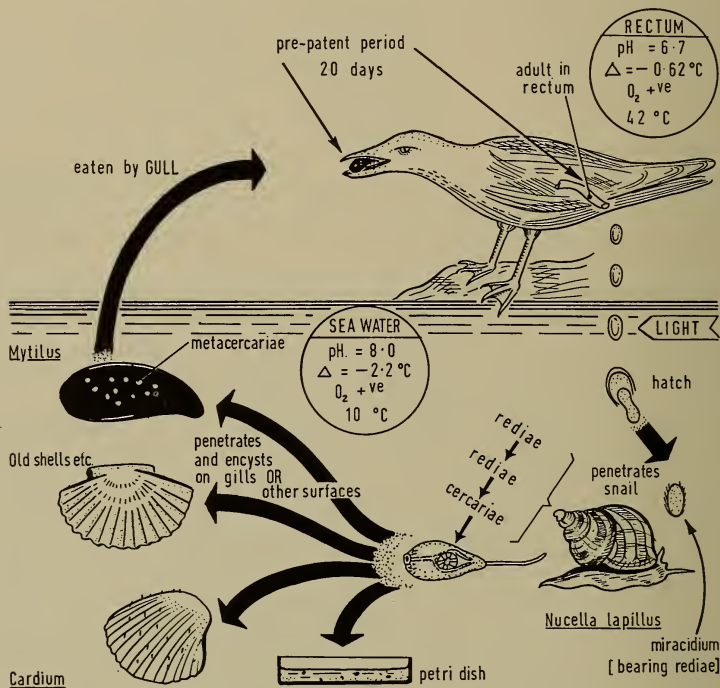


FIG. 70. *Parorchis acanthus*—life cycle and some physiological factors relating to it (original).

Excystment takes place in the duodenum, and the released metacercariae make their way to the rectum or into the bursa Fabricii. A wide range of shore birds are infected, but experiments have failed to grow worms in guinea pigs, rats or mice. The low level of available food material in the rectum or bursa Fabricii probably accounts for the long prepatent period of about fifteen days (the exact time has never been determined)

and strikingly demonstrates the effect of food supplies on the maturation process in trematodes. In contrast, duodenal forms such as the strigeid *Diplostomum phoxini* (p. 209), which live in a highly nutritive region of the intestine, can mature within four days, although morphologically the metacercariae appear as advanced as those of *P. acanthus*. These differences may be due to basic differences in potential growth rates but are equally likely to be related to the limitations of the food supply of the habitat.

### 15.22 Other Echinostomes

*Echinostoma revolutum*. A common parasite of ducks and geese, and occasionally man; it occurs in the rectum or caeca. Numerous molluscan intermediate hosts: *Stagnicola palustris*, *Helistoma trivolvis*, *Physa gyrina*, *P. occidentalis*, *P. ocularis*, *Lymnaea stagnalis*, *L. attenuata*, *L. pereger*, *L. swinhoeri*, *Planorbis tenuis*. Metacercariae occur in various molluscs.

*Echinostoma ilocanum*, A parasite of the Ilocanos of the Philippines. Metacercariae occur in the large operculated snail *Pila luzonica*, which is eaten raw.

*Echinostoma lindoense*. A parasite of man in the central Celebes regions. Metacercariae in lake mussels.

## 15.3 Family Heterophyidae

Very small (sometimes less than 0.5 mm) egg-shaped trematodes, usually parasitic in fish-eating animals. They show a striking morphological peculiarity in the possession of a *gonotyl* or genital sucker, a retractile sucker-like structure which assists in copulation and which may either be incorporated in the ventral sucker or lies on one side of it. The life cycle closely resembles that of the Opisthorchiidae, the eggs when laid containing miracidia. Hatching does not take place until the eggs are ingested by the molluscan host.

### 15.31 Type Example: *Cryptocotyle lingua*

definitive hosts:	fish-eating birds and mammals
situation:	intestine
molluscan host:	<i>Littorina littorea</i>
second intermediate host:	numerous marine fish

*Morphology*. Apart from the possession of the gonotyl already referred to, this fluke shows no special morphological peculiarity. Its anatomy is shown in Fig. 71.

*Occurrence*. The natural hosts of this widely distributed parasite are fish-eating birds, especially those inhabiting sea coasts (e.g. greater black-backed gull, herring gull,

lesser black-backed gull, slavonic grebe, night heron, common tern, razor bill, and kittiwake; maturation is not achieved in the duck). One of the characteristics of the fluke is its unspecificity regarding definitive hosts and many laboratory mammals such as rats, cats and guinea pigs may be infected, but the degree of resistance varies enormously. Wild rats probably serve as important reservoir hosts in nature.

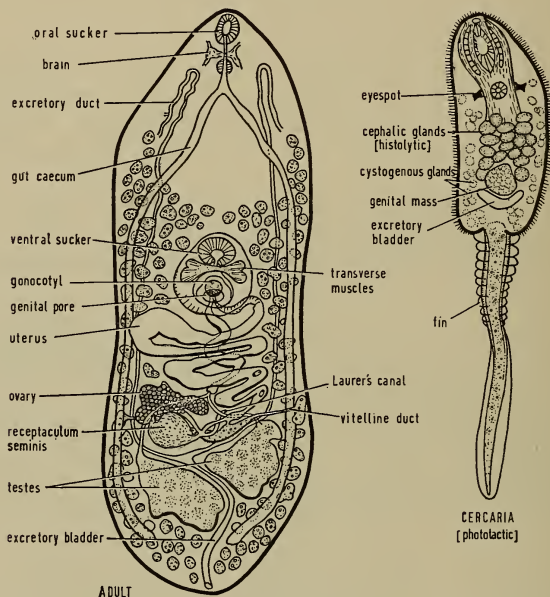


FIG. 71. *Cryptocotyle lingua*—morphology of adult and cercaria after Brown, 1950).

*Life history* (Fig. 72). The adult flukes live deep between the villi of the small intestine, and are capable of producing, rapidly, vast numbers of eggs. These embryonate in sea water at 20°C. in about ten days. Hatching has not been observed, and probably only takes place on ingestion by the molluscan host. Miracidia penetrate into the common shore periwinkle *Littorina littorea* and pass through the usual sporocyst and redia stages.

Wiley and Gross (1957) have shown that due to the destruction of part of the liver of snails *Littorina littorea* by the larval stages of this species, there is a release of liver pigment which gives the foot a distinct brown colour. Infected snails can thus readily be detected from uninfected snails by observing them through glass as they crawl up the side wall of a glass vessel. The pigment concerned is insoluble in water, but soluble in a variety of organic reagents and belongs to the carotenoid group; it shows a marked absorption peak near 450 millimicrons and a lesser one at about 660.

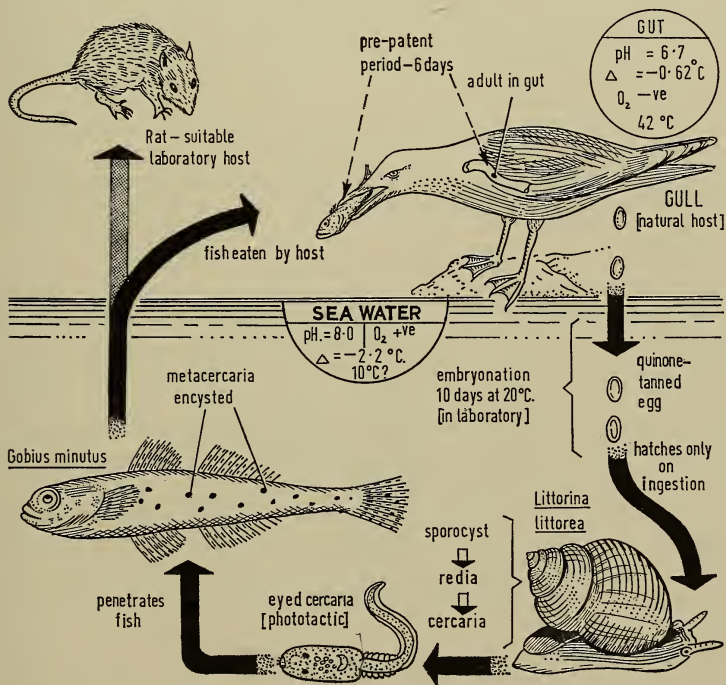


FIG. 72. *Cryptocotyle lingua*—life cycle and some physiological factors relating to it (original).

The released cercariae have prominent eye spots, are markedly phototropic and well supplied with both cystogenous and histolytic glands. Infected snails can release enormous numbers of cercariae, 3,000 a day have been reported and there is a well authenticated case of a single periwinkle releasing a total of several million cercariae



over a period of five years (Meyerhof and Rothschild, 1940). The cercariae will readily penetrate into a variety of shore fish (e.g. cunner, gudgeon, etc.). Although the tissues can be penetrated at any site, the process requiring some two hours to accomplish, cercariae show a predilection for the cartilage fin rays and frequently appear there in enormous numbers. The cyst secreted by a cercaria is thin and flexible and later becomes surrounded by connective tissue as a result of a tissue reaction by the host. As with many trematode cysts in fish, an orange-red or black pigment develops in the cyst site. Encysted metacercariae may grow to some two to three times their initial size and many remain viable for several years.

Two cyst coats are produced by the cercariae. The outer cyst coat is proteinaceous and the inner, lipoidal; the outer digests in pepsin, and the inner remains unaffected. Both cysts are readily weakened by the effect of digestive enzymes *in vivo*, and metacercariae are released in the upper region of the small intestine some six to twelve hours after ingestion.

### 15.32 Other Heterophyidae

Many species of Heterophyidae are characterised by the wide range of hosts in which they can mature. A number of species can mature in man and may occur in a variety of unusual tissue sites such as the heart or other viscera where they deposit eggs and may do damage. They reach these sites through infiltration into the general circulation via the intestinal walls. Two intestinal species may be regarded as 'normal' intestinal parasites of man since, although occurring in a wide range of mammalian hosts, they do not wander from the intestinal location. The commonest of these are:

*Heterophyes heterophyes*. A tiny fluke whose size varies with the definitive host, common in cats, dogs and man in the Far East, Egypt and Palestine. The molluscan host is *Pirenella conica*, and the fish host usually the mullet, *Mugil cephalus*, in which metacercariae of low tissue organisation are found; the genital sucker is present in the metacercariae.

*Metagonimus yokogawai*. A common parasite of dogs and cats in the Far East, the Northern Provinces of Siberia and the Balkan States. Other laboratory hosts are readily infected. In human infections, large numbers of flukes sometimes occur, the location being the duodenum. The snail, intermediate hosts are species of Thiaridae (formerly Maleniidae). The fish hosts are *Richardsonium hakuensis* and *Plectoglossus altivelis*, beneath the scales of which the metacercariae encyst.



### 15.4 Family Troglotrematidae

These are medium or fairly large 'fleshy' trematodes with poorly developed suckers. The absence of strongly developed suckers clearly limits the distribution of these flukes to environments where currents such as occur in the intestine, or other mechanical stresses, are lacking. They are almost entirely limited to cyst-like spaces in various mammalian organs such as lungs, frontal sinuses, skin and kidneys. There is no species readily available for laboratory study; the species described below is the best known.

#### 15.41 Type Example: *Paragonimus Westermanni*

definitive hosts:	man, carnivores and rodents
location:	lungs
molluscan hosts:	snails of families Thiaridae and Hydrobiidae
second intermediate hosts:	crabs of genera <i>Eriocheir</i> and <i>Potamon</i>

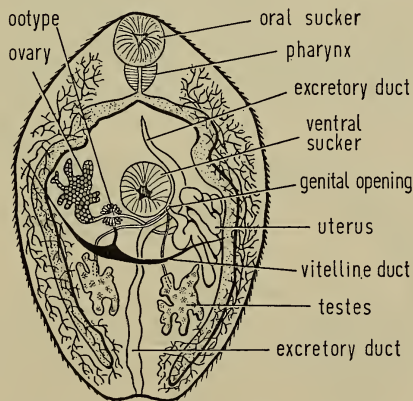


FIG. 73. *Paragonimus westermanni*—morphology (after Chandler, 1955).

**Morphology.** The adult flukes are rather egg-shaped in general form, thick and reddish in colour. The internal anatomy (Fig. 73) presents no unusual feature.

**Life cycle** (Fig. 74). Although the majority of adult specimens occur in the lungs, their digestive system can cope with a variety of food materials, for they occur in a wide range of tissues such as brain, liver, spleen, intestinal wall, eye, muscles or even kidneys. The occurrence in this wide range of habitats suggests that man may not be the natural

host. The lung cysts in which the worms most commonly occur usually contain at least two flukes. The eggs are freed into the bronchial tubes and pass out with sputum, but they may appear in the faeces as a result of being swallowed.

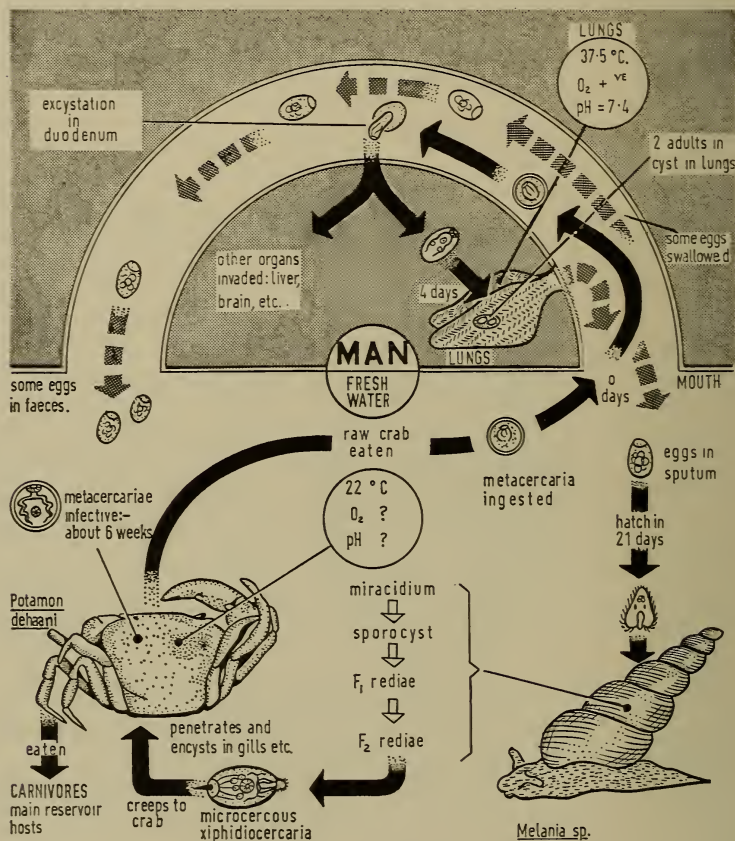


FIG. 74. *Paragonimus westermani*—life cycle and some physiological factors relating to it (original).

Eggs embryonate in three weeks at 22°C. and probably hatch by a light-triggered response. The molluscan hosts vary with the locality: in the Orient, they are species of the family Thiaridae mainly *Thiara granifera*, *Semisulcospira libertina* and *S. amurensis*. Sporocyst and first and second generation of rediae occur, giving rise to microcercous cercariae (p. 58) which on account of their short tails are unable to swim but creep or are carried along with the current. These penetrate a number of fresh-water crustaceans, especially species of *Potamon* and *Eriocheir* in the Orient, *Cambarus* in the United States and *Pseudotellphusa* in South America, in which they encyst in various sites such as gills, muscles, heart and liver. Encysted metacercariae are not immediately infective but require to undergo a further maturation phase. The time required for this phase to take place will depend on the nature of the encystment site (as well as the temperature of the host). In sites with a highly nutrient level such as liver or muscle, the metacercariae become infective in about six weeks. In other less nutrient sites, maturation takes longer.

On ingestion by the mammalian host, cysts are digested in the duodenum and the freed metacercariae bore through the intestinal wall into the body cavity to reach the pleural cavity in about four days and the lungs in about fourteen to twenty days. *Nanophyetus salmincola* (Syn. *Troglorema salmincola*). An interesting trematode whose metacercariae transmit the rickettsia-like organism *Neorickettsia helmintheca* causing 'salmon poisoning' to dogs. The adult flukes are parasites in fish-eating mammals, the metacercariae encysting in salmon. The life cycle resembles *Paragonimus*. Although other carnivores and rodents are infected, 'salmon poisoning' develops in *Canidae* only (dogs, foxes and coyotes). Immunity is developed after recovery.

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## CHAPTER XVI

### DIGENEA:

### FAMILY SCHISTOSOMATIDAE

#### 16.1 General Account

Members of the family show morphological and physiological peculiarities which set them apart from all other trematodes. Firstly, they are dioecious, the male bearing the female in a ventral canal, the *gynecophoric* canal, and secondly, they live in the blood stream of warm-blooded hosts, being the only trematodes to do so.

Only one other family shows sexual dimorphism, the Didymozoidae, cyst-dwelling trematodes of fish, and only two other trematode families, the Spirorchidae and the Aporocotylidae occur in the blood stream; these are parasites of cold-blooded hosts. Although the life cycles of these families are known, in general they have not received much attention and are not considered further here.

In mammals, the peripheral blood stream as an environment is relatively poor in carbohydrates and protein break-down products of low molecular weight. On the other hand, the portal system, which carries intestinal break-down products from the duodenum, is rich in glucose and amino acids, so that together with the protein available in the plasma and blood cells, it would represent an environment of a level sufficient to satisfy the metabolic demands of an egg-producing trematode. That this is so is evidenced by the efficiency with which a number of species of schistosomes grow and reproduce there.

Although schistosomes are easily maintained in the laboratory, and are probably more studied than any other helminth parasite in the world, many aspects of their physiology and even their morphology are imperfectly known.

Three species of blood flukes infect man and the disease they cause (schistosomiasis), is now the most important of helminth origin, causing untold misery to some hundreds of millions of people annually. Other species of schistosomes occur in a wide range of mammals and birds.

*Suitable laboratory species.* One of the species attacking man, *Schistosoma mansoni* (Table

22), is readily maintained in the laboratory in mice, hamsters and cotton rats, and because of its wide usage has been selected as the type example here. On account of its non-pathogenicity to man, the rodent parasite *Schistosomatum douthitti*, which has been less studied, is however, a safer and often more convenient form for routine laboratory investigations. Its life cycle will be discussed later (p. 194).

TABLE 22

THE RELATIVE SUSCEPTIBILITIES OF COMMON LABORATORY  
MAMMALS TO INFECTIONS BY *SCHISTOSOMA MANSONI*  
(data from Stirewalt, Kuntz and Evans, 1951)

Host	Maturing per cent	Fatality per cent	With eggs in faeces per cent
Hamster . . . . .	31·8	71	++
Mouse . . . . .	22·1	49	+
Cotton rat . . . . .	17·2	12	+
Guinea pig . . . . .	6·4	9	o
Cat . . . . .	10-45	o	o
White rat . . . . .	2·6	1	o
Rabbit . . . . .	0-18	o	o
Dog . . . . .	o	o	o

### 16.2 Type Example: *Schistosoma mansoni*

definitive hosts:	man, mice, hamsters (Table 22)
location:	mesenteric veins
molluscan hosts:	species of the genus <i>Biomphalaria</i> (Table 23)

#### *Morphology.*

*General.* The adult worms are beautifully adapted to life within blood vessels. The male is actually flat but has the sides of the body rolled ventrally to form a gynecophoric canal bearing the long narrow body of the female. The cuticle of the male is covered with minute papillae. In the female these only occur at the anterior and posterior ends, the middle region being mainly held in the gynecophoric canal of the male. Oral and ventral suckers are present, the latter being larger and more muscular in the male than the female and serve to maintain the position of the worms within the blood vessels against the circulatory current.

Recent electron microscope studies of the schistosome cuticle have shown it to be considerably vacuolated (Gönnert, 1955a), suggesting that cuticular as well as intestinal absorption is possible.

The alimentary canal is unusual in that the paired intestinal caeca rejoin about the



middle of the body to continue as a single winding tube ending blindly posteriorly. The nervous, muscular and excretory systems present no unusual features.

*Reproductive system.* Except for the lack of a muscular cirrus, all the usual trematode genitalia are present.

*Male.* There are 6-9 testes with efferent ducts leading to a vas deferens which swells to form a seminal vesicle opening by a non-muscular cirrus tube into the genital pore

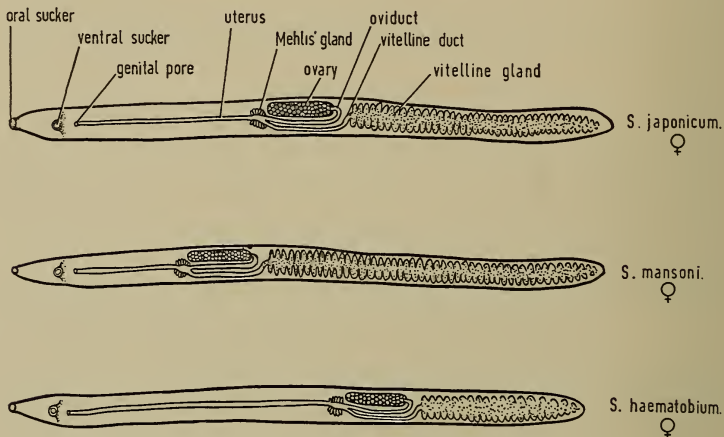


FIG. 75. Comparative morphology of female genitalia in schistosomes of man (after Blacklock and Southwell, 1954).

situated just posterior to the ventral sucker. The diploid number of chromosomes is  $2n=16$ .

*Female.* The vitellaria occupy the posterior part of the body and alternate on either side of a single median vitelline duct. The latter leads into a well developed ootype with which the uterus and oviduct bear the usual relationship (Fig. 75). The proximal part of the oviduct acts as a receptaculum seminis. A Laurer's canal is lacking. The shape of the egg, which varies diagnostically in the three human species, appear to be determined by the shape of the ootype (Fig. 76); this varies strikingly in the different species (Gönner, 1955b).

The schistosome egg shell, although giving positive histochemical tests for pro-



teins and phenols gives only weakly positive results for phenolase, and may be a modified form of sclerotin (p. 144).

### *Life cycle* (Fig. 77).

*Adult worms.* The adult worms normally reside in the mesenteric veins; but the females require to be carried there by the males (see p. 194). The females lay only one egg at a time, a process requiring some 7–8 minutes, and they retreat slightly along the venule after each egg is deposited. The worm is so orientated when oviposition is taking place that an egg is laid with the spine pointing backwards; this spine tends to catch on the intima of the blood vessels and prevents the egg being swept away by the blood current.

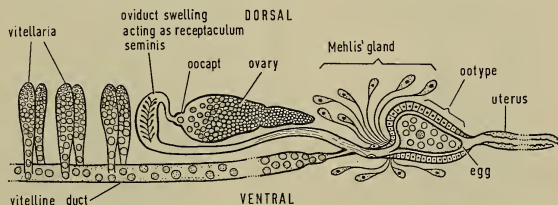


FIG. 76. Morphology of female genitalia of *Schistosoma mansoni* (after Gönner, 1955).

When laden with eggs, the vessels eventually rupture so that eggs find their way into the mucosa and submucosa, and hence into the intestinal lumen to the faeces. Some eggs are enclosed in scar tissue and destroyed, and others are carried by the venous current to the liver and spleen, and even to other organs such as the lungs, brain and spinal cord. A pair of worms produce about 160–190 eggs daily. In laboratory infections in mice, pairs of worms are frequently found in the lungs, as happens occasionally in man also.

*Fate of eggs.* Eggs which fail to reach the intestinal lumen are eventually phagocytosed or, more rarely, calcified, in the various tissue sites in which they occur. Eggs when laid, are unembryonated and require 6–7 days to embryonate in the tissues. After an infection has been patent for some time the majority of eggs in the liver contain miracidia. The total life of a miracidium within an egg is about 20 days, after which time the miracidium degenerates, the egg shell bursts and becomes phagocytosed by giant cells.

*Hatching.* Some of the factors affecting hatching have been considered by Stander (1949, 1951). Eggs when passed in faeces or obtained experimentally from macerated livers, contain mature miracidia. Hatching is inhibited by an osmotic pressure over about  $\Delta = -0.5^{\circ}\text{C.}$  and by the absence of light. On dilution of faeces by water, in the

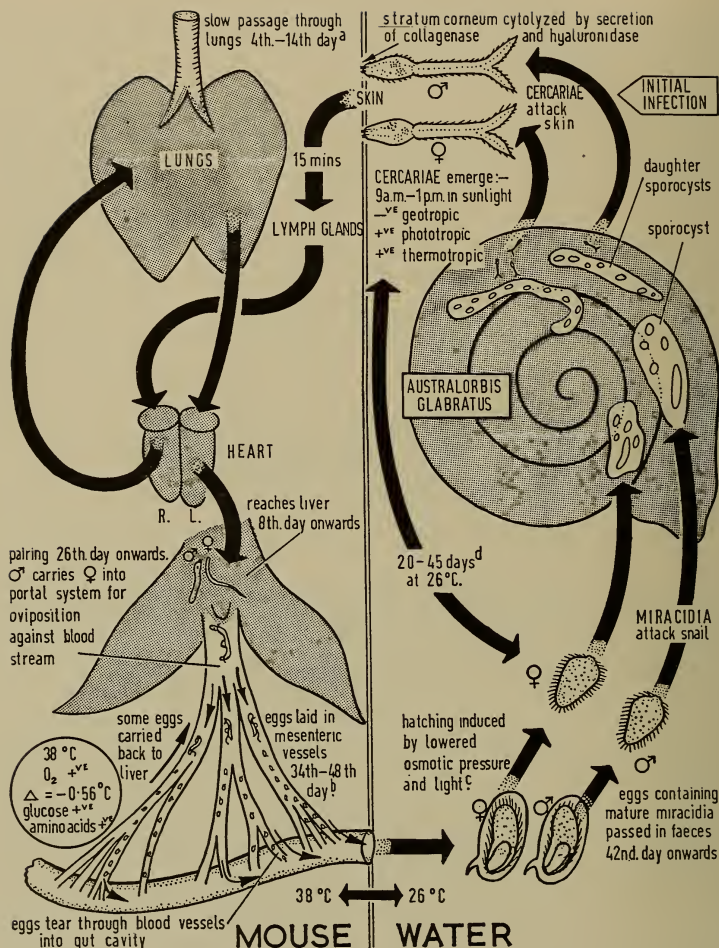


FIG. 77. Life cycle of *Schistosoma mansoni* in the albino mouse. Details of developmental times taken from the following: (a) Olivier (1952); (b) Standen (1953a); (c) Standen (1951); (d) Standen (1949) (original).

presence of light, hatching rapidly occurs. The hatching mechanism is thus a dual one. In liquids of low osmotic pressure, water is presumably taken in, but the egg capsule is unable to burst until exposed to light. This may be due to the action of a light-released substance, possibly an enzyme. *Schistosoma* eggs hatch at an optimum temperature of 28°C. and the process is almost completely inhibited at 4°C. and at body temperature 37°C. (Fig. 54). There is no operculum, so that if enzymes are concerned in the hatching they presumably attack and weaken the entire shell and not merely the operculum-cementing substance as probably happens in many other trematodes.

*Miracidium*. A miracidium of *S. mansoni*, which is fairly typical of the schistosomes attacking man, is shown in Fig. 78. Miracidia are markedly phototropic and move to the bright side of the container in which hatching occurs. This behaviour may be used

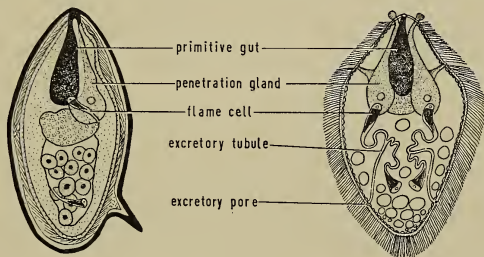


FIG. 78. *Schistosoma mansoni*—mature egg and miracidium (after Faust, 1939).

to obtain concentrations of miracidia. There are two pairs of penetration glands (an anterior and an antero-lateral pair), a primitive gut, two pairs of flame cells, and germinal cells. This miracidium is distinguishable both morphologically and physiologically from those of other schistosomes of man, but the differences are slight.

*The intra-molluscan phase.* Roughly about a dozen species of snails have been incriminated as intermediate hosts for *S. mansoni*.

They all belong to the family Planorbidae (Table 23). The main species in Egypt is the ditch and pond species *Biomphalaria (Planorbis) boissyi*; in other parts of Africa and Madagascar, it is the river species *B. pfeifferi* which is concerned. In S. America, the main carriers are *Australorbis glabratus* and *Tropicorbis centimetralis*.

A miracidium has sufficient food reserves to enable it to swim actively for 24–36 hours, after which time it dies if a favourable snail is not reached. The cilia are not lost during penetration of a snail, but these are used to assist in a passage deeper into the

tissues. A single smooth-walled sporocyst develops which produces daughter sporocysts; these make their way into lymph spaces and so to the lymph sinuses of the digestive gland. Here they grow and when mature contain the typical furcocercariae which escape from the snail. In *Australorbis glabratus*, these intramolluscan stages develop at an optimum temperature range of 26–28°C. (Table 29). At 26°C., the time required for

TABLE 23

THE MAIN INTERMEDIATE MOLLUSCAN HOSTS OF  
*SCHISTOSOMA MANSONI*

Molluscan host	Distribution
* <i>Biomphalaria</i> ( <i>Planorbis</i> ) <i>boissyi</i>	. Egypt.
<i>Biomphalaria</i> ( <i>Planorbis</i> ) <i>pfeifferi</i>	. Most parts of Africa; Madagascar.
* <i>Australorbis glabratus</i>	. South America; West Indies.
<i>Biomphalaria</i> ( <i>Planorbis</i> ) <i>sudanica</i>	. Central African Territories
<i>Tropicorbis centimetralis</i>	. South America.

Those marked \* are suitable for laboratory culture

development from miracidium to emergence of cercariae varies from 15–75 days in *A. glabratus* and 15–100 days in *B. boissyi*.

Standen (1949) found that in *B. boissyi*, 32 per cent of the snails shed more cercariae between 40th–55th days of incubation than in any other period for the species; in *A. glabratus*, 31 per cent commenced shedding between the 25th and 35th days. By the 45th day, 88 per cent of *A. glabratus*, but only 48 per cent of *B. boissyi*, had commenced cercarial discharge. Thus, in order to recover 90 per cent of potential cercaria shedders, *A. glabratus* need only be kept for 45–50 days after infection, but *B. boissyi* must be kept for 70–75 days.

Emergence of cercariae is periodic, and in nature tends to occur in direct sunlight between 9 a.m. and 2 p.m., but this process is inhibited or partly inhibited by temperatures lower than about 21°C.

*Cercariae*. It is claimed that minor differences exist between the cercariae of the three schistosomes of man, although some workers believe that no reliable criteria for their differentiation exists. The covering of both body and tail is provided with minute spines. The ventral sucker is well developed; a 'head organ' serves as an anterior sucker. Ventral to the latter is a small oral opening leading into a capillary oesophagus ending in a bilobed pocket representing the gut anlagen. A small cluster of genital cells occurs posteriorly to the ventral sucker. The excretory system consists of two pairs of flame cells, the most posterior cells occurring in the proximal region of the tail. A pair of collecting tubules join into a median bladder and a median posterior collecting tubule extends down the tail, bifurcating at the furcae and opening at the end of each furca by a pore. *Invasion and maturation*. Human infection is brought about by bathing or wading in

infected waters. In laboratory animals, infection is artificially induced by placing the animals in water heavily infected with cercariae.

Cercariae locate a host by chance and there is no evidence that chemotaxis is involved. Penetration of the skin is effected by a combination of lytic outpourings from the penetration glands and mechanical movement. The anterior glands empty first and the posterior glands later, usually after the stratum corneum has been penetrated. Hyaluronidase has been detected in the secretions, but powerful proteinases must also be present. Cercariae attach themselves to the epidermis, destroy the stratum corneum and stratum malpighi by enzyme action, and penetrate at first at right angles and then later parallel to the skin surface (Standen, 1953b). The tail is lost soon after penetration. The dermis may be reached as rapidly as 10 minutes, and cercariae have been detected in the lymphatic system within 20 minutes. There is a slight difference in the pattern of penetration obtained in heavy and light cercarial infections.

Cercariae reach the venous circulation either directly or via the lymph vessels. The early stages of development are very imperfectly known both in the mouse and man, but it is believed that the schistosomulae pass through the lungs after 4-14 days. They then migrate to the intrahepatic portal vessels (in about 8 days at the earliest), where the early stages of maturation occur. In mixed infections with male and female cercariae (see p. 194) paired worms may be found in the mesenteric and portal veins after about 26 days, but the majority leave on about the 30th day (Standen 1953a). In animals infected with male cercariae only, the males develop normally but there is a tendency for worms to delay their migration from the liver to the portal system. The addition of females to an already established male infection greatly increases the proportion of worms migrating into the portal system. In mice, full sexual maturity is reached by about the 28th day. In normal mixed infections in mice, eggs appear in the faeces within 5-7 weeks.

*Choice of laboratory host.* The relative susceptibilities of laboratory mammals to infections of *S. mansoni* are listed in Tables 22 and 25. Some practical details for maintenance are given in Table 28. Although mice, hamsters and cotton rats are satisfactory hosts, each has its advantages or limitations (Stirewalt, Kuntz and Evans, 1951). For particular lines of investigation, therefore, the host most suitable must be selected. Thus, although the hamster has the highest percentage of maturing schistosomes and the largest number of available eggs in the faeces, its high death rate is a disadvantage. Where large well-developed adult worms are required, the cotton rat is the most suitable choice. The common white rat is, curiously enough, an unsuitable host, developing resistance terminating in self cure approximately six weeks after infection.



*Occurrence in man.* *S. mansoni* can now be regarded as one of the most important parasites of man, particularly in Egypt. It is a worm that is associated with perennial irrigation which provides excellent breeding grounds for the snail hosts. It is especially common in the lower Nile delta, the Upper Sudan and Venezuela, but it is also widely distributed in other parts of Africa and tropical America.

### 16.3 Other Species Attacking Man

#### 16.31 *Schistosoma haematobium*

*Geographical distribution.* This species is prevalent in Africa, especially in the Nile Valley where up to 95 per cent of the local rural population may be infected. As with *S. mansoni*, its distribution runs parallel to irrigation projects and in general wherever local conditions are favourable for the molluscan vectors. It also occurs in the islands of Mauritius and Madagascar and has been reported from India.

*Morphology.* In general, it resembles *S. mansoni*, but differs from it in a number of minor points:

- (a) both male and female worms are longer.
- (b) there are only 4 large testes (6-9 in *mansoni*).
- (c) the ovary is in the posterior third of the body (Fig. 75).
- (d) the uterus contains large numbers of eggs (*mansoni*: 1-4 eggs only).
- (e) the eggs possess a terminal spine and are passed in urine, rarely in faeces.

TABLE 24

#### THE MAIN INTERMEDIATE MOLLUSCAN HOSTS OF *SCHISTOSOMA HAEMATOBIMUM*

Molluscan host	Distribution
<i>Bulinus truncatus</i> . . .	North and South Africa; S.W. Asia; the Mediterranean area; E. African highlands.
<i>Bulinus (Physopsis) africanus</i> .	Equatorial African lowlands.
<i>Bulinus cernicus</i> . . .	Mauritius.
<i>Planorbis cornutus metidjensis</i> .	Portugal.
<i>Bulinus laticostatus</i> . . .	Madagascar.

*Habitat.* Mature worms live *in copula* mainly in the tributaries of the inferior mesenteric veins and the females deposit their eggs in the walls of the urinary bladder, ureters or urethra, through which they slowly infiltrate into the urine; a few eggs may reach the rectum and appear in the faeces. As in *S. mansoni* some eggs are carried back to the liver and sometimes to other viscera.

*Life cycle.* Very similar to that of *S. mansoni*. Eggs hatch rapidly on dilution of urine and on exposure to light. Molluscan vectors are listed in Table 24. Times for the various



developmental stages to appear are very incompletely known. Sexual maturity is reached in about 4–5 weeks, but eggs may not appear in the urine until 10–12 weeks, or even considerably later.

*Laboratory hosts.* The susceptibility of numerous laboratory hosts has been studied in detail by Kuntz and Malakatis (1955) with the following results:

Albino mice, hamsters, monkeys, baboons: good hosts for experimental studies (Table 25).

TABLE 25  
INFECTION OF MONKEYS WITH THE SCHISTOSOMES OF MAN

Species	<i>S. mansoni</i>	<i>S. haematobium</i>	<i>S. japonicum</i>
<i>Cercopithecus</i> sp. . . . .	+	+	—
<i>Cercopithecus aethiops</i> . . . .	+	+	—
<i>Cercopithecus sabeus</i> . . . .	+	+	—
<i>Macacus cynomolgus</i> . . . .	—	+	+
<i>Macacus philippinensis</i> . . . .	—	—	+
<i>Macacus mulatta</i> . . . .	+	+	—
<i>Macacus rhesus</i> . . . .	—	—	+

+ =infection positive; — =infection negative (based on data by Kagan, 1953)

Albino rats, cotton rats, guinea pigs: poor hosts; the numbers of parasites are characteristically reduced in the early stages.

Rabbits, dogs and cats: almost completely refractory to infection.

Of these hosts, the hamster is the best all-round host for general studies; in it, the schistosomes reach the veins in the vicinity of the urinary bladder as well as those of the lower levels of the large intestine. In the primates, the conditions of involvement are comparable to those occurring in man.

### 16.32 *Schistosoma japonicum*

*Geographical distribution.* Confined to the Far East, the infected areas being China, Japan, Formosa, the Philippines and Palee district of Celebes.

*Morphology.* Differs from *S. mansoni* in a number of minor points (Fig. 75).

(a) male longer and narrower.

(b) integument free from tubercles (except for minute spines on suckers and gynecophoric canal).

(c) testes 6–7, characteristically compressed into a single column.

(d) the ovary is in the middle of the body.

(e) uterus has many eggs.

(f) eggs with only short lateral spines and passed in faeces.

*Habitat.* The adult worms live in *copula* chiefly in the superior mesenteric veins and deposit some 500 eggs daily (one hundred times the daily output of *S. mansoni*) in the intestinal walls. Eggs infiltrate through the tissues and are passed in the faeces. As with other species, eggs are carried to the liver and may reach the other viscera.

*Life cycle.* This is not significantly different from *S. mansoni*. The molluscan vectors are listed in Table 26. The organism is non-specific in its choice of hosts, and rodents and

TABLE 26  
THE MAIN INTERMEDIATE MOLLUSCAN HOSTS OF  
*SCHISTOSOMA JAPONICA*

All species belong to the family Amnicolidae

Molluscan host	Distribution
<i>Oncomelania nosophora</i> . . . . .	Japan, China, Celebes
<i>Oncomelania hupensis</i> . . . . .	China
<i>Oncomelania quadrasi</i> . . . . .	Philippines
<i>Oncomelania formosana</i> . . . . .	Formosa

domestic animals serve as important reservoir hosts. Field mice, dogs, cats, buffaloes, oxen, pigs and deer have all been incriminated. Sexual maturity is reached in about four weeks and eggs may appear in faeces as early as five weeks.

### 16.33 Development in unisexual infections

In mice infected from female cercariae of *S. mansoni* or *S. japonicum* only, the growth and maturation of adult females is greatly stunted, and virtually no migration into the mesenteric veins occurs. The addition of males to an all-female infection has a striking effect. Pairing commences as soon as the males are mature, the females develop normally and migration of the paired worms takes place as in normal infections. It is likely that the failure of the females to migrate into the portal and mesenteric veins is due to the physical weakness of the females and their inability to migrate against the blood stream unless carried by the larger and more powerful male. The failure of the genitalia to mature in the absence of the male suggests the existence of a possible male stimulation factor although this has never been proved. It is remarkable that in *Schistosomatium douthitti*, both males and females develop normally in unisexual infections in mice.

#### 16.4 Type Example: *Schistosomatium douthitti*

natural definitive hosts:	numerous rodents (e.g. field mice, deer, mice, albino mice, musk rats)
suitable laboratory hosts:	mice, hamsters (Table 27)
location:	hepatic portal system
molluscan hosts:	<i>Lymnaea</i> spp., <i>Physa</i> spp., <i>Stagnicola</i> spp. (Table 28)

Although the human blood flukes are readily maintained in the laboratory in experimental hosts, the danger of an accidental infection is always present. Moreover, certain governments lay down strict

quarantine laws regarding the passage and importation of pathological material, so that the establishment of laboratory cultures may be difficult. For these reasons, the maintenance of the rodent species *Schistosomium douthitti*, which is non-pathogenic to man, offers many advantages. This schistosome has essentially the same life cycle as those species parasitising humans, and it is now extensively used in many laboratories for experimental work on schistosomes. An excellent account of its laboratory maintenance is given by Kagan, Short and Nez (1954).

TABLE 27  
DEVELOPMENT OF *SCHISTOSOMATIUM DOUTHITTI* IN  
DIFFERENT HOSTS

Host	Development	Host	Development
Musk rat . . .	normal	Albino mouse . .	normal
Lynx . . . . .	normal	Rat . . . . .	abnormal
Deer mouse . .	normal	Cat . . . . .	abnormal
Field mouse . .	normal	Rabbit . . . . .	abnormal
Hamster . . . .	normal	Monkey . . . . .	Immunity developed in three weeks

#### 16.41 Morphology (Fig. 79).

*Male*: length, 1.9–6.3 mm. The body is divided into two distinct parts, a *prebody* which is flattened and occupies two-fifths of the body, and a *hind-body* occupying the remaining three-fifths and forming a gynecophoric canal. There are from 14–16 testes situated between the intestinal caeca of the anterior end of the hind body. Each testis follicle opens via a short vas efferens into a single median vas deferens leading to a seminal vesicle. The latter, together with the cirrus, is enclosed in a cirrus pouch. Spines cover most of the body.

*Female*: length, 1.1–5.4 mm. The essential female organs are present: ovary, oviduct, vitellaria, vitelline duct, seminal receptacle, ootype, Mehli's gland and uterus. A Laurer's canal has not been described. The arrangement of the genitalia is shown in Fig. 79. The ootype is oval in shape and lined with large refractive cells. As in other species of schistosomes, the spines on female worms are limited in distribution, covering the lateral edges of the dorsal and ventral surfaces, and extending from the anterior part of the body posteriorly to the beginning of the ovary. The oral sucker also has spines.

When *in copula*, a female worm is held within the gynecophoric canal, its dorsal surface against the ventral surface of the male with only its anterior end protruding. *Digestive system*. The oesophagus is characterised by the presence of conspicuous oesophageal glands presumably secreting digestive enzymes for the breakdown of the blood. The oesophagus bifurcates into two intestinal caeca which have numerous lateral diverticula; these caeca unite near the posterior end of the body. Various anomalies

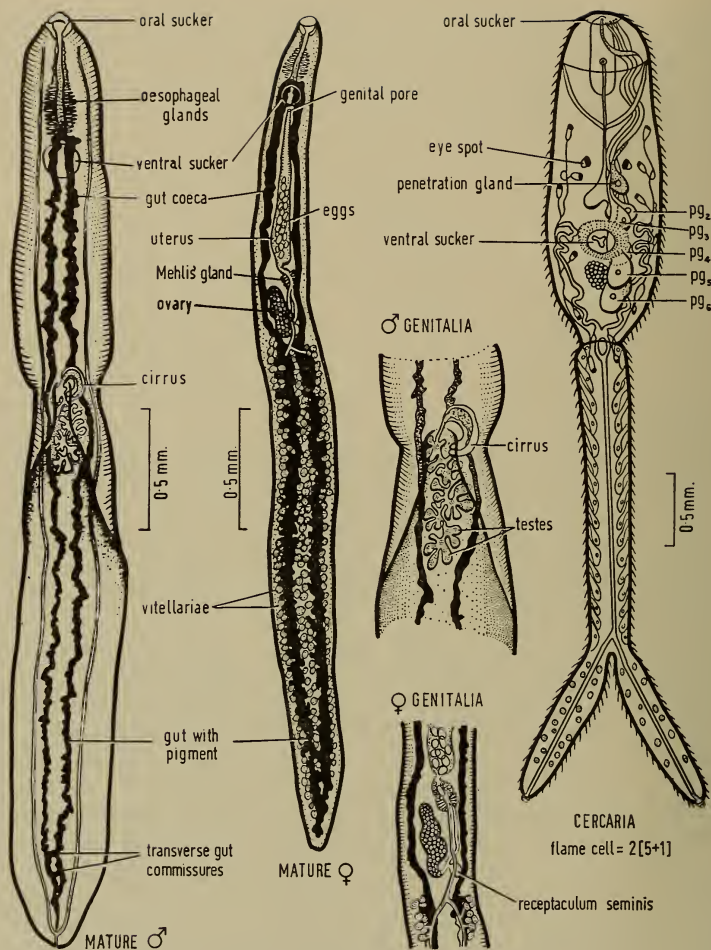


FIG. 79. Morphology of the rodent schistosome *Schistosomatum douthitti* (after Price, 1931).

of the gut can occur. The digestive system is better developed in the female than in the male, the intestinal caeca being wider and holding more blood. This dimorphism is clearly related to the nutritional requirements of the female and the enormous demands made on its metabolism by the egg-producing genitalia.

*Egg.* The egg of *S. douthitti* which measures  $42-80 \mu \times 50-58 \mu$ , is smaller than that of the schistosomes of man (e.g. *S. mansoni*— $150 \mu \times 60 \mu$ ) and unlike them is free from a lateral spine.

*Habitat.* The adults live *in copula* within the mesenteric veins.

### 16.42 Life Cycle

This has been described in detail by Price (1931) and El-Gindy (1950-51). Some practical details are summarised in Table 29.

In spite of lacking a spine, the eggs are able to penetrate the intestinal wall and pass out with the faeces. The majority of eggs, however, pass to the liver where they collect and from which they may be obtained in the laboratory by maceration. Eggs hatch when the medium (faeces, urine or saline) is diluted, provided light is present.

Released miracidia are negatively geotropic and positively phototropic and this property may be used to separate them from a suspension of eggs and tissue in water, either by the use of the light-beam technique or the side-arm flask method. In the latter method, macerated liver is placed in a flask with a side arm and allowed to settle after several changes of 0.85 per cent saline. After the supernatant is clear, the saline is decanted and replaced by tap water up to the mark first. It is then filled to the brim by rapidly pouring more water *down the side arm*; this leaves the side arm clear. The bulb of the flask is covered by a cloth or a bag and the side-arm placed 12-15 in. from a light source. Miracidia, which first appear in about 5-10 mins., swarm into the side arm and are easily visible against a dark background with the naked eye. (Kagan, Short and Nez, 1954). A fresh batch of miracidia may usually be obtained by decanting and replenishing the flask with fresh water.

The morphology of the miracidium does not differ significantly from that of *S. mansoni*.

TABLE 28

#### MOLLUSCAN INTERMEDIATE HOSTS OF THE RODENT SCHISTOSOME, *SCHISTOSOMATIUM DOUTHITTI* IN THE UNITED STATES

\**Lymnaea stagnalis appressa*  
*Lymnaea stagnalis perampla*  
*Lymnaea stagnalis sanctaemariae*  
*Lymnaea stagnalis lilliana*  
*Lymnaea stagnalis jugularis*

\**Lymnaea palustris*  
*Physa ancillaria parkeri*  
*Physa gyrina elliptica*  
*Stagnicola exilis*  
*Stagnicola emarginata angulata*

Those marked \* are suitable for laboratory culture

*Molluscan stages.* The natural snail hosts from which cercariae of this species have been obtained in the United States are listed in Table 28. The percentage of natural infection

is small, within the range of 3-7 per cent (Kagan, Short and Nez, 1954; El-Gindy, 1950). On account of easier availability, *Lymnaea stagnalis appressa* and *L. palustris* are the snails of choice, and both are easily maintained in an aquarium at 22-24°C. Experimental infections usually result in snail infections of 25-80 per cent.

Although snails of all ages can be infected, young snails (3-10 weeks old) are more readily infected than older ones. As with the schistosomes of man, there is no redia stage in the developmental cycles within the snail, miracidia giving rise to mother sporocysts which produce daughter sporocysts, which release cercariae. The incubation period is dependent on temperature; at a maintenance temperature of 22-24°C., cercariae emerge within 37-52 days after the initial infection. As in other schistosomes, cercariae from a single miracidium produce worms of one sex (but see p. 200).

TABLE 29

SUMMARY OF PRACTICAL DETAILS FOR THE LABORATORY MAINTENANCE OF  
*SCHISTOSOMA MANSONI* AND *SCHISTOSOMATIUM DOUTHITTI* IN MICE

(figures based on Kagan, Short and Nez, 1954, and Standen, 1949)

	<i>Schistosoma mansoni</i>	<i>Schistosomatium douthitti</i>
Definitive laboratory host . . . . .	mouse	mouse
Molluscan laboratory host . . . . .	<i>A. glabratus</i>	<i>L. palustris</i>
Maintenance temperature (snail breeding) . . . . .	24-25°C.	22-24°C.
Maintenance temperature (snail infection) . . . . .	26-28°C.	22-24°C.
Age of snails for infecting . . . . .	10-14 weeks	3-10 weeks
Exposure time of snails to miracidia . . . . .	5-6 hours	20-24 hours
Number of miracidia per snail . . . . .	15	3-5
Time for cercariae to emerge . . . . .	15-75 days	37-52 days
Number of cercariae per mouse for infection . . . . .	130-150	50-75
Time of attainment of sexual maturity . . . . .	28 days	10-12 days
Time for eggs to appear in faeces . . . . .	5-7 weeks	26 days

*Cercariae*. The morphology (Fig. 79) of the cercaria differs from that of the *S. mansoni* only in minor respects. Thus, there are two well-developed eye-spots in *S. douthitti* (absent in human schistosomes) and the cercariae are markedly phototropic. There are six pairs of flame cells, five in the body and one in the tail. The formula is thus  $2(5+1)=12$ , in contrast to the schistosomes of man in which the formula is  $2(3+1)$ . The behaviour pattern of emergence is, however, different. In *S. mansoni*, possibly correlated with the habits of their hosts, shedding of cercaria takes place in direct sunlight between 9 a.m. and 2 p.m., but in *Schistosomatium douthitti* shedding only occurs in the evening or at night (Fig. 80). The reasons for this pattern of periodic behaviour is unknown (Olivier, 1951). Although laboratory-infected snails tend to produce smaller numbers (500-1,500), naturally infected snails have been known to produce up to 5,000 cercariae in one evening.



*Development within the definitive host.* The natural definitive hosts in the United States are the musk rat (*Ondatra zibethica*), the deer mouse (*Peromyscus maniculatus*), and the meadow mouse (*Microtus pennsylvanicus*). In the laboratory, hamsters and albino mice are the most suitable hosts. In rhesus monkeys, although sexual maturity is reached, immunity develops within three weeks after which time the worms are killed or destroyed. In rats, cats and rabbits development is poor or abnormal.

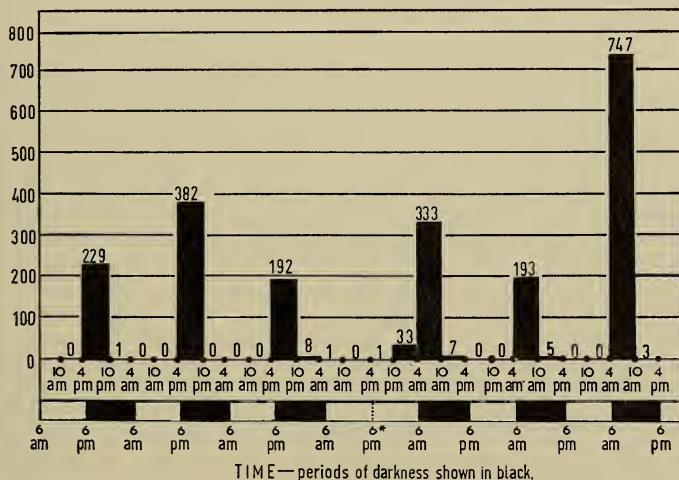


FIG. 80. Effect of light on the emergence of cercariae of *Schistosomatium douthitti*. Note how the pattern changes when, at the point \*, the snails are kept in the dark between 4 and 10 a.m. (after Olivier, 1951).

As far as is known, the mechanism of skin penetration resembles that of the schistosomes of man, and the five pairs of penetration glands probably produce hyaluronidase and collagenase. The route of migration from the skin to the portal system is uncertain; cercariae may reach the blood system directly by piercing capillaries, or entry may be via the lymph system. According to one view (El-Gindy, 1950) the liver is reached through the pleural cavity. The schistosomulae develop within the vessels of the liver from which they migrate into the mesenteric veins about 10–11 days after infection; by about the 13th day practically all worms are in the extra-hepatic portal vessels, and are either sexually mature, or nearly so. All worms are usually mature by 20 days.

This rapid maturation contrasts strangely with that of *S. mansoni* where sexual maturity requires more than twice as long (28 days). This marked discrepancy has at least partly a nutritional basis. In *S. mansoni* the passage through the lungs is slow (4-14 days) and the majority of schistosomulae do not reach the liver before about the eighth day after infection, which means that they do not come into contact with the veins of the liver carrying their rich supply of amino acids and carbohydrates until that time. In *S. douthitti*, on the other hand (and in *S. japonicum*) passage through the lungs is rapid and the liver is reached about the fourth day, so that the rich nutriment of the liver is available earlier than in *S. mansoni*. There is also evidence that *S. douthitti*, which causes severe lung lesions, is feeding on particulate food in the lungs, as worms removed from the lungs contain brown granular material in their gut. In specimens of *S. mansoni* from the lungs, the gut is usually colourless. Hence in the lungs, *S. douthitti* may be obtaining more nutriment than *S. mansoni*.

The growth rates of *S. mansoni*, *S. douthitti* and *S. japonicum* as found by Olivier (1952) are compared in Fig. 81. Note that in *S. mansoni* the growth rate after about 16

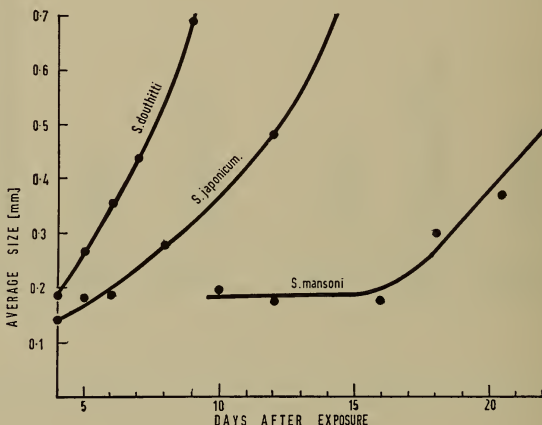


FIG. 81. Comparative growth of *Schistosomatium douthitti*, *Schistosoma mansoni* and *Schistosoma japonicum* in mice. Maturation of *S. douthitti* is achieved in about 11 days (after Olivier, 1952).

days rises suddenly and thereafter compares favourably with that of *S. japonicum* and *S. douthitti*.

Worms have a life span of some 1-1½ years in favourable hosts such as mice or hamsters.

*Unisexual infections.* A single miracidium produces cercariae which when matured give rise to worms of one sex only. When pooled cercariae from several snails are used, male

worms (55 per cent) slightly outnumber female worms, a situation reported in bisexual infections for other schistosome species also.

In striking contrast to *S. mansoni*, the female worms of which will only develop if carried into the portal system by the males (p. 191), both sexes of *S. douthitti* reach maturity in the normal time (10–12 days) regardless of the presence of the other, Interspecific ‘crosses’ with other schistosomes have been successful in some cases, and female *S. mansoni* crossed with male *S. douthitti* have reached maturity. In such cases, it is believed that the eggs develop parthenogenetically, the females merely using the males to carry them into the portal blood stream. Hermaphrodite specimens of females with immature testis follicles have been reported.

Eggs which develop parthenogenetically hatch normally, but the miracidia produced are slightly smaller than those from fertilised eggs and less infective to snails. Uniparental cercariae are likewise slightly smaller than normal, and again show less infectability. Worms developed from uniparental cercariae give rise to both male and female worms (in an approximate ratio of male/female=4/1). It can be concluded, therefore, that females are digametic and produce both male- and female-determined eggs.

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## CHAPTER XVII

### DIGENEA:

### STRIGEIDAE, DIPLOSTOMATIDAE, PARAMPHISTOMATIDAE

#### 17.1 Families Strigeidae and Diplostomatidae

These families are conveniently considered together as they constitute a group of trematodes loosely referred to as 'strigeids' or 'holostomes'. In general, they are distomes in which the body (Fig. 82) is clearly divided by a constriction into a flattened or cup-shaped portion, which acts essentially as an adhesive organ, and a posterior cylindrical portion containing the genitalia. A large 'holdfast' or tribocytic organ provided with histolytic glands is usually present. The life cycles in general resemble those of schistosomes to which, on the basis of the morphology of the cercaria (fork-tailed) and the flame-cell pattern (1+1) in the miracidium, they appear to be closely related. The morphological difference between the two families is slight, the anterior region being more flattened in the *Diplostomatidae* than in the *Strigeidae*. Both groups produce peculiar metacercariae, known as '*diplostomulum*' and '*tetracotyle*' respectively, which occur in definite tissue sites and undergo morphological development slightly in advance of that found in a cercaria. Species of *Diplostomulum* occur in the eye, brain and spinal cord of fishes and amphibia. They lack cystogenous glands, and do not form a cyst wall, but can move actively in the host tissues. On the other hand, species of *Tetracotyle* occur both in invertebrates and vertebrates and form definite cyst walls. In many cases only the larval forms are known and may be frequently given characteristic names.

The nomenclature has been confusing in the past. The name *Diplostomulum* is now used for larval forms and the name *Diplostomum* for the adult. Thus the specific name given to a larva of the *Diplostomulum* group will disappear once its adult stage is known. *Diplostomulum spathaceum*, for example, is the larval stage of *Diplostomum spathaceum*.

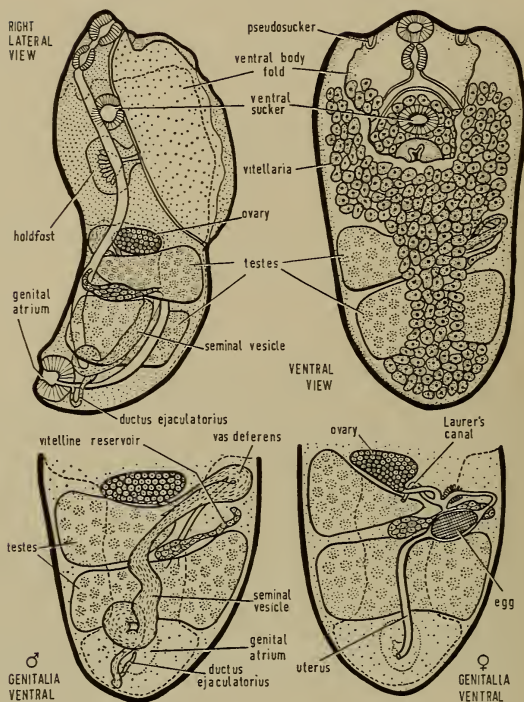


FIG. 82. *Diplostomum phoxini*—general morphology of adult from the intestine of gulls (after Rees, 1955).

### 17.11 Type Example : *Diplostomum phoxini*

definitive hosts:

*Mergus merganser merganser*, *Anas platyrhyncha*, *Cairina moschata*

location:

duodenum

molluscan hosts:

*Lymnaea pereger*, *L. auricularia*

second intermediate host:

*Phoxinus phoxinus* (European minnow), larvae in brain

**Occurrence.** The larval stages of *Diplostomulum phoxini* are amongst the commonest of trematode larval stages available for laboratory study in the British Isles. The brains of



minnows are usually 100 per cent infected, sometimes heavily. Rees (1955, 1957) has given detailed morphological descriptions of adult and larvae.

*Morphology.* The body shape is typically 'strigeid' with a flattened oval, anterior region with leaf-like edges, the whole serving as an adhesive organ. The anterior extremity (Fig. 82) is trilobed, the central region being occupied by the oral sucker and between it and the two lateral lobes are two *pseudosuckers*. In feeding, the fluke holds on to the surface by means of its pseudosuckers, an action which leaves the mouth free to browse on the semisolid content of the intestine. The cuticle is covered by minute spines which do not appear to penetrate the surface. There is a well-developed holdfast provided with glands, the secretions of which are believed to be histolytic in nature. The alimentary canal has a prepharynx, a pharynx and a well-developed pair of intestinal caeca. The arrangement of the genitalia which, except for the vitellaria, all occur in the non-flattened posterior region, is clear from Fig. 82. A few specific points of interest may be noted:

*Male.* The testes are large and occupy the anterior two-thirds of the posterior region. Sexual amphitopy is common, the anterior testis lying on the right or left side. There is no cirrus, cirrus sac or prostate glands, the ejaculatory duct opening into the posterior genital atrium. The latter is a deep cavity surrounded by a special arrangement of muscles, the whole being superficially sucker-like but not so clearly defined.

*Female.* The female genitalia present no unusual features. The vitelline cells are extensive and form three lobes, the anterior two extending nearly to the base of the pharynx. The eggs are relatively large for so small a worm; only 1-5 are found in the uterus at a time.

#### *Life cycle* (Fig. 83).

The early stages of the life cycle are very imperfectly known. The eggs presumably hatch in water, after embryonation in the presence of light, and penetrate snails. Only *Lymnaea pereger* (in Great Britain) and *Lymnaea auricularia* have so far been implicated. The polyembryonic stages within the snails have not been described.

*Cercaria.* For the purpose of experimental work, it may be important to be able to distinguish this cercaria from others (such as that of *D. spathaceum*, also common in *L. pereger*).

There is a circum-oval spineless area around the mouth, which is slightly sub-terminal; in this area are the openings of the four penetration-gland ducts. Just in front of the mouth are a group of fine spines pointing forwards. The ventral sucker is provided with a double row of alternating spines.

# AXENIC CULTURE



pH = 7.4  
 $\Delta = -0.56^{\circ}\text{C}$   
 41  $^{\circ}\text{C}$   
 $\text{O}_2 \pm \text{ve}$   
 SHAKEN

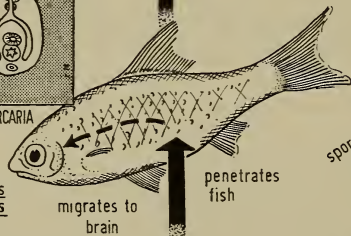
yolk  
 albumen  
 gelatine

removed aseptically



METACERCARIA

*Phoxinus phoxinus*



migrates to brain

penetrates fish

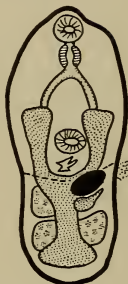


furcocercaria

FISH  
 pH. = 7.4 ?  
 $\Delta = -0.56^{\circ}\text{C}$   
 $\text{O}_2 = -\text{ve}$   
 10.  $^{\circ}\text{C}$ .

# ADULT DIPLOSTOMUM [in bird duodenum]

normal eggs  
 appear—96 hrs



GUT  
 pH = 6.7  
 $\Delta = -0.62^{\circ}\text{C}$   
 $\text{O}_2 = -\text{ve}$   
 41  $^{\circ}\text{C}$

quinone-tanned shell

eggs in faeces

Definitive hosts  
 Natural — Gulls  
 Laboratory — Ducks

WATER  
 pH. = 7.0  
 $\text{O}_2 = +\text{ve}$   
 10.  $^{\circ}\text{C}$ .

hatches

MIRACIDIUM released

LIGHT

penetrates snail



*Limnaea pereger*

sporocyst  
 $\downarrow$   
 mother sporocyst  
 $\downarrow$   
 cercaria

SNAIL  
 pH = ?  
 $\Delta = -0.22^{\circ}\text{C}$   
 $\text{O}_2 = -\text{ve}$   
 10.  $^{\circ}\text{C}$ .

FIG. 83. Life cycle of the strigeid *Diplostomum phoxini* and some physiological factors relating to it. For details of *in vitro* culture, see p. 417 (original).

The nature of the penetration-gland secretions has not been determined but, as in other species (p. 191), are probably hyaluronidase and a collagenase.

The flame-cell formula (Fig. 84) is:

$$2[(1a + 1b + 2) + (3 + 4a + 4b^1 + (4b^{11*} + 4b^{11**}))] = 16.$$

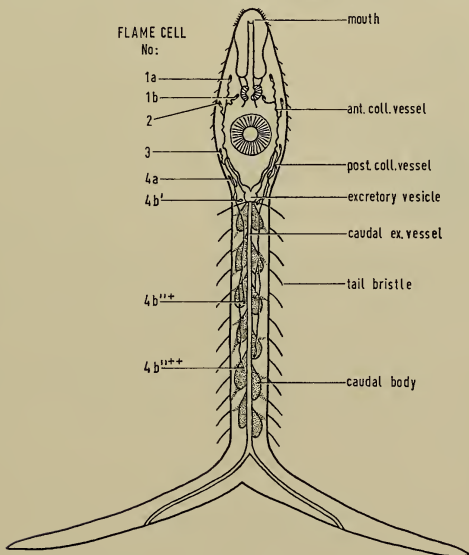


FIG. 84. Cercaria of *Diplostomum phoxini*, showing application of flame-cell formulae (after Rees, 1957).

There are six pairs of caudal bodies in the tail. The function of these is uncertain, but it is believed they may give buoyancy to the cercaria. The tail also bears twelve bristles.

On emergence from the snail, cercariae swim for some time, but eventually come to rest hanging, head downwards from the surface of the water with their furcae wide apart; the position taken up is of diagnostic importance.

*Penetration of minnow.* Cercariae easily penetrate the thin skin of minnows, lose their tails and rapidly make their way to the brain, probably via the blood stream, where slight haemorrhages are observable after a recent attack. They accumulate particularly

in the fourth ventricle, aquiductus sylvii, optic lobes, third ventricle, lobi inferiores, sometimes in the corpora striata and under the epithelium over certain areas of the brain and spinal cord.

*Development of metacercaria.* Within the brain, the tailless cercariae become metacercariae (*Diplostomulum phoxini*) which require a further period of some 28 days (at 10–15°C.) before they reach maximum development.

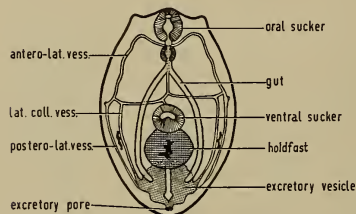


FIG. 85. Metacercaria of *Diplostomulum phoxini* from brain of minnow, *Phoxinus phoxinus* (modified from Rees, 1955).

A fully developed metacercaria (Fig. 85) superficially bears the body shape of an adult. Internally it shows a number of advances over the cercaria, namely (a) the gut caeca are well developed, (b) the flame cells increase in number, and (c) the secondary excretory system, often referred to as the 'reserve bladder system', develops. The gut caeca usually remain empty, although sometimes containing pigment. For the most part, metacercariae merely absorb

soluble food material within the brain tissues, but if severe haemorrhages have taken place, blood and cerebral fluid may be taken into the gut. The sixteen flame cells in the cercaria give rise, by repeated division, to 104 flame cells. The cell formula of the metacercaria being:

$$2 [(4+4+4) + (4+4+4+4+4) + (4+4+4+4+4)] = 104.$$

The flame-cell system is often difficult to make out in the living condition on account of the reserve bladder system, the terminations of which are associated with calcareous corpuscles. The system is similar in the related species, *D. spathaceum*.

Nothing is known as to how long metacercariae will survive within the brain tissues. Although the larvae require a sojourn of some 28 days in the brain to become infective, they continue to grow slowly in size without further tissue differentiation. Lack of further differentiation is interpreted as being due to the environment being unable to supply sufficient nutriment to satisfy the requirements of the organogeny stage of development (p. 419).

*Maturation of metacercariae.* When infected fish are eaten by birds, the metacercariae become firmly attached to the duodenal mucosa and rapidly reach maturation. Domestic ducks serve as suitable experimental hosts, although other anserines such as *Mergus merganser merganser* serve as natural definitive hosts. The times required for the various

phases of maturation have been worked out and histochemical criteria established (Bell and Hopkins, 1956; Bell and Smyth, 1958). These phases are as follows:

- 0-24 hrs. Characterised by intensive mitosis corresponding to the segmentation stage of an embryo.
- 24-36 hrs. Organogeny well advanced. Ovaries and testes recognisable in aceto-orcein squashes. Early 'comma' spermatids recognisable in testes.
- 36-40 hrs. Appearance of mature active spermatozoa in the testes and receptaculum seminis.
- 72 hrs. Vitellaria well developed and giving positive histochemical reactions (diazotisation and catechol) for egg-shell precursors (p. 144).
- 84 hrs. Eggs present in uterus.

*D. phoxini* may be cultured axenically, almost to maturity (p. 415).

## 17.12 Other Strigeids

Many other species of strigeid metacercariae are common parasites of cold-blooded vertebrates, especially fish. The nervous system or sense organs are commonly involved. In the British Isles, a common species is *Diplostomum spathaceum*, whose cercariae are shed from *Lymnaea pereger*; the adult flukes occur in gulls of the family Laridae. Cercariae penetrate the skin of fish and amphibia and migrate forwards to the lenses where they become metacercariae. Gulls act as convenient laboratory hosts. A related species, *D. flexicaudum*, occurs in the British Isles and the United States (Van Haitsma, 1930) and numerous other species are known. In general, the life cycle follows the pattern of *D. spathaceum*.

## 17.2 Paramphistomatidae

Members of this family, commonly referred to as *amphistomes*, are long thick forms, almost circular in transverse section and characterised by the presence of a large posterior sucker, often enormously developed. This latter feature is present in the cercaria also (Fig. 58). The eggs are unusually large. They are parasites of fishes, amphibians, reptiles, birds and mammals; the best known species are from domestic animals.

*Paramphistomum cervi*. Occurs in the reticulum and rumen of ruminants, especially sheep, goats and cattle, mainly in Africa, but also in other countries. The general shape is pear-shaped and it is pale red in colour. The life cycle is similar to that of *Fasciola hepatica*, with the metacercariae encysting on vegetation. Numerous molluscan intermediate hosts have been incriminated. *P. scotiae* and *P. hiberniae* have been reported from Scotland and Ireland respectively.

*Gastrodiscoides hominis*. This parasite occurs commonly in pigs in India, but occurs frequently in man, especially in localised areas such as Assam, where 46 per cent of the people have been reported to harbour it. The worm inhabits the caecum and large intestine, a relatively uncommon location for a trematode. The life cycle is imperfectly known, but is probably similar to *Fasciola hepatica*.

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## CHAPTER XVIII

# PHYSIOLOGY OF TREMATODES

The physiology of trematodes has been much less studied than that of cestodes or nematodes, probably on account of the difficulty of obtaining suitable experimental material. Only the metabolism of the blood fluke, *Schistosoma mansoni*, has been investigated in detail; experiments on *Fasciola* have been somewhat conflicting and unsatisfactory. Some detailed respiratory studies have also been made on the microphallid trematode *Gynaecotyla adunca*. Bueding and Most (1953) and von Brand (1952) have reviewed knowledge of this field.

Before considering the physiology of trematodes, it is as well to review certain features typical of the group which directly or indirectly impose certain limitations or requirements on their physiology.

(a) All trematodes possess at least one sucker, which means that they are brought into intimate contact with host tissues. The definitive habitat must thus present a suitable surface to which attachment can be made and on which feeding can take place.

(b) Without exception, they possess a well-developed alimentary canal usually with a muscular pharynx. This kind of system is especially suitable for ingesting semi-liquid or viscous food such as intestinal contents, mucus, blood and bile, and with the exception of a few neotenic forms (p. 151) distribution of adults is limited to habitats, chiefly in vertebrates, where such food materials are available.

(c) They lack both the tough outer cuticle of nematodes and the absorptive surface of cestodes; their external covering is well developed and usually spiny, but apparently relatively permeable to small molecules such as glucose.

(d) Their egg capsules are delicate structures, incapable of withstanding desiccation, and normally require water for development.

(e) Their life cycles are complex (excepting the Monogenea) and always involve a molluscan intermediate host, sometimes a second intermediate host and, more rarely, a third intermediate host. The physico-chemical conditions and the nutritional levels of the different environments encountered may thus vary profoundly, so that striking

changes in the metabolism of various stages may be expected. This is clearly demonstrated in the respiration of larvae (p. 216).

### 18.1 Chemical Composition

As is stressed elsewhere (p. 269), the chemical analysis of parasitic organisms seldom provides significant information unless it is related to the nutritional state of the host, and the degree of maturity of the parasite. Thus the carbohydrate content of an intestinal parasite may vary within considerable limits, depending on whether the host has been actively feeding or starving before autopsy. This point is illustrated particularly by experiments with cestodes (p. 269) but the general principle applies to trematodes also.

Only two species, *Fasciola hepatica* and *Schistosoma mansoni*, have been subjected to detailed chemical analysis, and even in the case of these species the data are very incomplete (Table 30). Chemical analysis data are usually expressed as a percentage of the dry weight. The dry/fresh weight ratio may vary appreciably with the maturity of the fluke. Thus in the case of *Echinostoma revolutum*, experimentally developed in chicks, the dry weight after 10 days was approximately 10 per cent and after 30 days 20 per cent.

**Carbohydrate.** As in other parasitic helminths, the main polysaccharide found in trematodes is glycogen, and that obtained from *Fasciola* is a highly dextro-rotary compound which does not appear to differ significantly from glycogens from vertebrate tissues. There is no evidence that the glycogen is linked with proteins, as in some cestodes.

**Lipids.** Only *Fasciola hepatica* has been studied in detail, and in this species the fat appears to be concentrated in the excretory vessels, and expulsion of fat droplets through the excretory pore has been described. The fat distribution in *Fasciola* may not be a representative pattern for trematodes, since in some other forms this type of fat excretion does not occur. In some larval trematodes, the excretory bladder is laden with fatty material. The metacercariae of *Bucephalopsis gracilescens* (Fig. 60), for example, have a large excretory bladder filled with fatty material, which in fresh preparations appears dark by transmitted light.

The lipid material in *Fasciola* consists quantitatively of phosphatids, unsaponifiable matter, unsaturated fatty acids, saturated fatty acids, in that order. The unsaponifiable fraction is a sterol, possibly cholesterol. The only unsaturated fatty acid identified with reasonable certainty is oleic acid and among the saturated acids only palmitic and stearic acids have been definitely reported.

**Proteins.** The protein constituent of trematodes is very imperfectly known and only for *Schistosoma mansoni* and *Fasciola hepatica* are figures available (Table 30). No detailed

TABLE 30

## CHEMICAL ANALYSES OF TREMATODES

A number of factors such as age, phase of development and nutritional condition of host must be taken into account for these figures to be of significant value.

Species	Dry matter as % fresh worm	Analysis (% dry wt.)			Reference
		Carbo- hydrate (glycogen)	Lipid	Protein	
<i>Schistosoma mansoni</i> (males)	.	—	—	50	Bueding and Koletsky (1950); Radke <i>et al.</i> (1957)
<i>Schistosoma mansoni</i> (females)	.	1.4-2.9	—	65	Bueding and Koletsky (1950); Radke <i>et al.</i> (1957)
<i>Fasciola hepatica</i>	.	3-5	—	58	von Brand (1952)
<i>Echinostoma revolutum</i>	.	15-21	12-13	—	Senger (1954)
<i>Gynaecotyla adunca</i>	.	—	—	—	Vernberg and Hunter (1956)
<i>Haematoloechus complexus</i>	.	4-28 (wet wt.)	—	—	Odlaug (1955)
<i>H. medioplexus</i>	.	0.37	—	—	
<i>Gorgodera amplicava</i>	.	0.81-0.92	—	—	
<i>Gorgoderina attenuata</i>	.	0.83	—	—	
<i>Clinostomum attenuatum</i> (metacercariae)	.	0.85	—	—	
<i>Crepidobothrium sapheua</i>	.	1.6	—	—	
	.	1.2	—	—	

analysis of the amino acid composition of these proteins has been made although such information is unlikely to reveal anything unusual. The eggs of many trematodes consist of quinone-tanned protein (p. 144), although in some species (e.g. *Schistosoma mansoni*, *Gorgoderia* sp.) another tanning system may occur. In *Fasciola* the cuticular spines give the same histochemical reaction as the egg shell, and are presumably sclerotin also. Attempts to isolate phenolic materials from *Fasciola* have been unsuccessful (Smyth and Clegg, 1959).

### 18.2 Water Relationships

The osmotic relationships of trematodes with their environments has been very little studied. This is surprising, for their complex life cycles may involve in turn passage through fresh or sea water (by the miracidium and the cercaria), an intermediate molluscan host, and often a second intermediate host (usually an arthropod or a vertebrate) before reaching the definitive host. A study of the osmotic changes encountered and how they are overcome might produce some interesting results. Both miracidia and cercariae of *Fasciola hepatica* face considerable osmotic changes on passing to or from tissue ( $\Delta = -0.58^{\circ}\text{C}$ . for cattle) to fresh ( $\Delta = 0^{\circ}\text{C}$ .) or sea water ( $\Delta = -2.2^{\circ}\text{C}$ .), or to snail tissues ( $\Delta = -0.22^{\circ}\text{C}$ . for *Lymnaea stagnalis*). In a miracidium, except for the simple flame-cell system, there is no special device for osmotic control. In a cercaria, on the other hand, the flame-cell system is more highly developed, and the excretory bladder is pulsatory in nature. In this respect its function resembles that of the excretory canals of nematode larvae (p. 296), and probably plays a major role in water elimination.

### 18.3 Nutrition

Trematodes resemble nematodes in possessing a well-developed alimentary canal, but differ in that this canal ends blindly. There is little *direct* evidence of the normal food requirements of trematodes although the nature of the food material may often be predicted on theoretical grounds. How far trematodes have retained the primitive enzyme systems present in their free-living platyhelminth ancestors is not known, but there is growing evidence that a well-developed digestive enzyme system exists in many species and that others have evolved specialised systems to cope with specific food materials in special environments.

The feeding habits have been most thoroughly studied in the case of *Fasciola hepatica* (in rabbits) by the use of radio-isotope techniques (Jennings *et al.*, 1954, 1955). The evidence suggests that it is blood which is ingested and not break-down products of blood excreted in bile.

The technique adopted was to inject blood, in which the red cells were labelled with  $P^{32}$  or the serum albumins with  $I^{131}$ , into control and infected rabbits. It was found that the level of radioactivity of the bile was low compared with that of the blood. After one hour, there was a large quantitative difference between the radioactivity of the worms and the bile, thus indicating clearly that blood was directly ingested.

The monogenetic trematode *Polystoma intergerrimum* likewise appears to feed mainly on blood from the capillaries in the frog bladder.

In schistosomes, the intestinal caeca contain a blackish-brown pigment derived from the digestion of ingested blood. Although the composition of this pigment has not been extensively studied, the evidence suggests it is identical with the malarial pigment (p. 92). The pigment is much more abundant in the female than the male, a fact which is taken to indicate that the female feeds more actively. This is the expected result, for the nutritional requirements of egg-production are much greater than those of sperm production. Schistosomes live in the hepatic-portal system and the blood in these vessels is rich in soluble food materials such as amino acids and monosaccharides. A certain amount of absorption of these soluble food materials through the cuticle may be possible.

Intestinal trematodes appear to feed largely, if not entirely, on food detritus, mucus and probably bacteria. The rate of development may be related to the definitive environmental site amongst other factors. Those high up the alimentary canal in the duodenum generally develop rapidly (*Diplostomum spathaceum* matures in the gut within 72 hrs., see p. 418), whereas those further down the intestine usually take relatively long periods. Thus *Parorchis acanthus* (p. 177) in the caecum or rectum may require three weeks to become mature. This generalisation may not hold true for all species, but the nutritional level of the environment undoubtedly plays a very significant role in determining the rate of maturation (see p. 22).

*Larvae.* Little is known concerning the nutritional requirements of the polyembryonic stages in the molluscan hosts. The predilection of the larvae for the digestive glands of the intermediate hosts is suggestive of high nutritional requirements during this phase. Released cercariae are non-feeding and their food reserves are rarely sufficient to enable them to remain active for longer than twelve hours.

#### 18.4 Respiration

*Eggs and larvae.* The eggs of the majority of trematodes embryonate in an aerobic environment (fresh water, sea water, moist grass or soil), but a few (*Schistosoma*, *Polystoma*) are already embryonated when laid. Little quantitative work has been done on the oxygen consumption of eggs.

*Cercariae.* Hunter and Vernberg (1955a) have used the delicate Cartesian diver respiro-

meter to measure the oxygen consumption of single cercaria of *Zoogonus rubellus*, a parasite of fish. It was mistakenly thought by these workers that the cercariae being used were those of *Gynaecotyla adunca*, a parasite of birds, and respiratory studies were made at higher temperatures (Table 31); later work showed that they were the cercariae of *Zoogonus rubellus*.

TABLE 31

OXYGEN CONSUMPTION OF VARIOUS STAGES IN THE LIFE HISTORY OF THE  
TREMATODE *GYNAECOTYLA ADUNCA*

Measurements made on individual organisms by means of a Cartesian diver respirometer. Stages marked thus \* were apparently identified incorrectly, and later shown to be those of a different species, *Zoogonus rubellus* (data from Hunter and Vernberg, 1955a; Vernberg and Hunter, 1956).

Stage of development	Temp. °C.	Microlitres/hr/mm <sup>3</sup>
*Free cercariae . . . . .	30.4	5.35
*3-4 days after penetrating crab . . . . .	30.4	not measurable
*Immediately prior to cyst formation . . . . .	30.4	0.159
Metacercariae (after encystment) . . . . .	30.4	5.62 × 10 <sup>-3</sup> (per worm)
Adults immediately after encystment . . . . .	23.6	0.130
Adults immediately after encystment . . . . .	30.4	0.290
24 hours after excystment . . . . .	30.4	0.153
48 hours after excystment . . . . .	30.4	0.120
72 hours after excystment . . . . .	30.4	0.104

Although the rate of respiration of schistosome cercariae has never been measured, it has been shown that cercariae of *S. mansoni* are extremely sensitive to lack of oxygen and die within one hour under anaerobic conditions.

*Adults.* Most of the work on adult-trematode respiration has been carried out on *S. mansoni*, except for experiments by Hunter and Vernberg (1955a, b), on *Gynaecotyla adunca*.

Although *S. mansoni* lives in a habitat of relatively high oxygen tension (Table 1) respiration appears to be largely anaerobic. At an earlier stage of development, two weeks before maturation, the dependence of the worm on aerobic metabolism for synthetic purposes appears to be greater. Under completely anaerobic conditions *in vitro*, *S. mansoni* survives for as long as five days (Bueding and Most, 1953).

In *S. mansoni in vitro*, the oxygen consumption was found to vary between 3.05 and 10.15  $\mu$ l per hr. per mg dry wt., if glucose was lacking, and between 6.71 and 12.8  $\mu$ l when the medium contained glucose (Bueding, 1950). The corresponding figures for the R.Q. were 0.58-1.61 and 0.76-1.12. Thus in the presence of glucose, the R.Q. approached 1.0. The respiration of *Schistosoma* is almost completely inhibited by a cya-



nine dye ( $1:10^6$ ), although the rate of glycolysis is unaffected. Agreed figures are not available for *Fasciola* as different techniques employed by different workers have given conflicting results, but respiration is inhibited by cyanide, azide and carbon monoxide. This definitely points to the participation of a heavy metal catalyst. Since the monoxide inhibition is reversible, the presence of an iron catalyst is indicated. This evidence is suggestive of the presence of the cytochrome system, but not conclusive proof that this system is operating. Only traces of cytochrome have been found in *Schistosoma* (Bueding and Charms, 1951).

The respiration of the microphallid *Gynaecotyla adunca*, has also been studied (Hunter and Vernberg, 1955a). This species is of especial interest since the adult can mature in *either* a bird *or* a fish host with a body-temperature difference of about  $20^{\circ}\text{C}$ . Unfortunately, measurements at  $40^{\circ}\text{C}$ . are difficult to make for technical reasons, and only figures for  $23.6^{\circ}\text{C}$ . and  $30.4^{\circ}\text{C}$ . are available (Table 31). The respiratory rate of the adults at  $30.4^{\circ}\text{C}$ . is more than twice that at  $23.6^{\circ}\text{C}$ .

## 18.5 Metabolism

### 18.51 Carbohydrate Metabolism

Like most other parasitic helminths, trematodes have a pronounced carbohydrate metabolism which has been extensively studied only in the case of *Schistosoma mansoni* (Bueding, 1950). All the evidence points to existence of the Embden-Meyerhof scheme of phosphorylating glycolysis although the detailed pathways have not been worked out. *In vitro*, *S. mansoni* utilises an amount of glucose equivalent to 15–26 per cent of its dry weight. The main end-product of the glucose metabolism is lactic acid. Over 80 per cent of the glucose consumed during respiration is converted to lactic acid. The rate of glucose utilisation and of lactic-acid production is the same under aerobic and anaerobic conditions. The bulk of the energy is thus obtained by glycolysis even under aerobic conditions. However, there is evidence that oxygen may be essential for normal maturation of the worm, possibly to permit the synthesis of an essential intermediate metabolite.

The following glycolytic enzymes have been shown to be present in extracts of schistosomes: several hexokinases, an isomerase, aldolase, triose phosphate dehydrogenase and lactic dehydrogenase; two adenosine triphosphatases have also been identified (Bueding and Most, 1953).

The fermentation processes in *S. mansoni*, resulting as they do mainly in the production of lactic acid, resemble those of cestodes (p. 278), the nematodes *Litomosoides carinii* and *Dracunculus insignis*, and the protozoans of the genera *Plasmodium* (especially *P. gallinaceum*). It is worth noting that not all helminths produce lactic acid in the same

form. *S. mansoni* produces (DL) lactic acid, whereas *L. carinii* produces only the L form. In *Fasciola hepatica*, lactic acid has not been detected as an end-product.

In the majority of helminths (pp. 276, 354) the period immediately following anaerobiosis is characterised by an increase in the rate of respiration. This is often referred to as repayment of an 'oxygen debt', and in those organisms in which an oxygen debt is incurred, it is assumed that some substances formed during the anaerobic period are retained in the tissue and become available for oxidation during subsequent aerobic periods. In *Schistosoma* (and in *Litomosoides*) this increase has not been observed.

### 18.52 Protein Metabolism

Virtually nothing is known regarding the protein metabolism of trematodes. Large-scale tissue synthesis takes place in the larval stages so that, for example, a single miracidium of *Schistosoma mansoni* may produce more than 200,000 cercariae. In adult trematodes, growth in size ceases once they become sexually mature, but nevertheless many species possess astonishing powers of protein synthesis as evidenced by the number of eggs produced, which in *Fasciolopsis buski* may be up to 25,000 daily. Nothing is known concerning the gross protein requirements or what amino acids are essential.

The influence of diet on egg-production may be strikingly demonstrated by experiments on the development of the strigeid *Diplostomilum phoxini* *in vitro*. This parasite normally matures in a bird gut, but may be matured *in vitro* at 40°C. in a medium of yolk + albumen (p. 417). Under these conditions, although eggs are formed, their protein shells do not give the normal histochemical reactions of a tanned protein (i.e. +<sup>ve</sup> to diazo reagents; +<sup>ve</sup> for phenolase). This result is suggestive of a deficiency in the protein diet, possibly due to specific amino-acid deficiency. When gelatine is added to the yolk-albumen mixture used as a medium, the histochemical reactions of the shell become more nearly 'normal', in that they approach those given by eggs from flukes matured in birds. This suggests that the gelatine supplies an 'essential' amino acid or acids, or other growth factor, absent in the yolk-albumen mixture. Since gelatine is a break-down product of the collagen in bone and since many quinone-tanned proteins are made up of collagen (e.g. the byssus thread of *Mytilus*), it may be that the egg-shell in trematodes similarly has a collagen basis which would account for the effectiveness of gelatine in boosting its egg-shell production (Bell and Smyth, 1958).

Little is known of the proteolytic enzymes in trematodes, either in the tissues or in the digestive tract. Arginase has been detected in the gut of *Fasciola*. Schistosomes can break down haemoglobin to globin and haematin but the enzyme system concerned has not been investigated. In *F. hepatica* homogenates, the presence of  $\alpha$ -ketoglutaric transaminase activity with a number of amino acids has been reported.

### 18.53 Lipid Metabolism

Nothing is known regarding the metabolism of lipids in trematodes. Various lipases have been detected in *F. hepatica* and *S. mansoni*.

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## CHAPTER XIX

# CESTODA: CESTODARIA

### 19.1 General Account

The Cestoda form a group of worms which, with a few exceptions, exhibit two striking morphological features; they possess an elongated tape-like body and they lack an alimentary canal. The elongated shape precludes them from habitats whose spatial arrangement provides no elongated axis. Thus, with the exception of the sub-class Cestodaria and a few neotenic larval forms in oligochaetes (e.g. *Archigetes*) adult cestodes occur only in tubular habitats, usually the alimentary canal, but occasionally in the bile or pancreatic ducts (e.g. *Stilesia*, *Atriotaenia*). These are habitats of high nutritional levels, a fact associated with the high rate of growth.

The larval habitat, on the other hand, shows a wide range of variation, and larvae can be found in almost any organ of both vertebrate and invertebrate hosts, although most larvae show a predilection for a particular site.

The lack of an alimentary canal separates cestodes markedly from trematodes and nematodes, and this feature, which is unique among Platyhelminthes, but shared with the Acanthocephala, dominates the physiology of the group, for unless the cestode cuticle is found to possess peculiar properties (see p. 226), only food materials of limited molecular dimensions can be absorbed.

Except in certain 'primitive' species, each tapeworm is a string of individuals having a complete set of reproductive organs in progressive degrees of sexual maturity and budding off from a body attached to the host tissue by a head or scolex. In one sub-class, the Cestodaria, and in the family Caryophyllacidae there is only one set of reproductive organs, and budding does not occur.

How this budding takes place, and indeed the whole question of tissue growth in cestodes, is very imperfectly understood. Until recently, somatic mitosis had never been satisfactorily demonstrated, and it had been suggested that the normal method of somatic cellular increase was by amitosis. Recent techniques, using colchicine and squash

procedures, have demonstrated that somatic mitosis follows the normal pattern of other metazoans (Smyth, 1957).

With the exception of *Hymenolepis nana*, which can develop directly, all cestodes require one or sometimes two intermediate hosts. These follow no general pattern, and can be either vertebrate or invertebrate, warm blooded or cold blooded.

**Classification.** The group may be divided into two sub-classes as follows:

**Cestodaria:** Cestoda which are not divided into segments and which contain only one set of reproductive organs; scolex lacking; 10-hooked larva (decacanth).

**Eucestoda:** Cestoda which become distinctly divided into segments or proglottids (except the Caryophyllaeidae), each containing a set of male and female reproductive organs; scolex usually present; 6-hooked larva (hexacanth).

## 19.2 Cestodaria

This is a group of worms of uncertain affinities, and although today they are restricted to a few species, they may have been more abundant in ancient times. The group is divided into two orders:

Order 1. Amphilinidea

Order 2. Gyrocotylidea

### 19.21 Amphilinidea

Amphilinids are unusual among the Cestoda in being parasitic in the body cavity. The growth and metabolic rates of these worms must be sufficiently low for their nutritional requirements to be satisfied by the constituents of the coelomic fluid.

**Type Example:** *Amphilina foliacea*

definitive host: *Acipenser* sp. (sturgeon)

intermediate host: *Gammarus* spp. or *Dikerogammarus*

location: body cavity [spp.]

**Morphology.** The body is leaf-shaped and creamy-white in colour. The arrangement of the genitalia is shown in Fig. 86. The male opening is in the middle of the posterior end. The cirrus is well developed and armed with ten hooks.

**Life history.** The thin-shelled eggs are elongated and bear a tiny stalk at one pole. When laid, they contain a curious ciliated larva termed a *lycophora* (Fig. 86). A mucous sub-

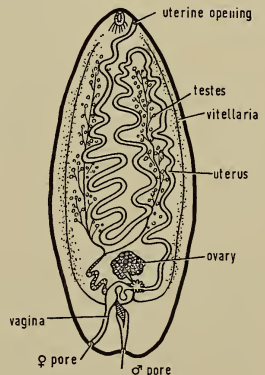


FIG. 86. *Amphilina foliacea*—from the body cavity of the sturgeon (after Bercham, 1901).

stance is secreted by the egg (probably from the well-developed glands of the lycophora) and swells when the egg comes into contact with water. This mechanism may enable the eggs to keep afloat and be more readily available for the intermediate hosts, which are fresh-water amphipods of the genera *Gammarus* and *Dikerogammarus*. The eggs, on ingestion, are ruptured by the crushing action of the mandible. The lycophora bores its way through the intestine into the haemocoel, and develops into a *proceroid* and later a *plerocercoid* larva, resembling the adult. Fish become infected by ingesting infected

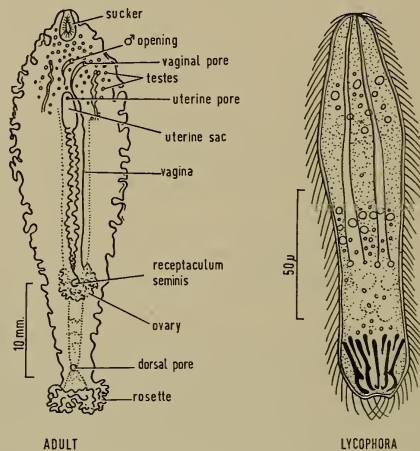


FIG. 87. *Gyrocyle ura*—adult and larva (after Lynch, 1945).

amphipods. The prepatent period in the definitive hosts is not known, but in view of the low nutritional level of the coelomic cavity is likely to be comparatively long. Eggs, when released, escape from the body cavity via the abdominal pores. Amphilinids can also penetrate the body wall of the host and protrude slightly through the pore formed, releasing their eggs into the water.

A related species, *A. paragonopora*, has been studied by Woodland (1923), but in general the life cycles are very imperfectly known.

Their occurrence within the body cavity rather than the gut has led some workers to suggest that amphilinids are only neotenic larvae whose definitive hosts have become extinct. This is a reasonable hypothesis in view of the fact that neotenic larvae are known to occur in at least one group of cestodes (*Archigetes*, p. 247) and they are also well known in trematodes (p. 151).



### 19.22 Gyrocotylidea

Adult gyrocotylids do not resemble amphilinids, but are clearly related to them, as witnessed by their lycophora larva. So far they have only been found as intestinal parasites of chimaerids, which occur only in deep water or in polar seas. The contracted body of a gyrocotylid is thrown into folds and bears a curious 'rosette' organ at its posterior end (Fig. 87). This organ is a funnel-shaped structure which passes into a duct which opens on the dorsal surface of the worm. The eggs require embryonation, but hatch in sea water, unlike the eggs of amphilinids. The intermediate hosts are not known. The best-known genus is *Gyrocotyle* (Lynch, 1945).

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## CHAPTER XX

# EUCESTODA: GENERAL ACCOUNT

### 20.1 Classification

Only four orders of cestodes are universally recognised: the Tetraphyllidea, Trypanorhyncha, Pseudophyllidea and Cyclophyllidea. Authors are not generally in agreement as to what other orders should be set up, and those marked with an asterisk \* are not considered here. The classification given below follows that of Hyman (1951). Order 1. *Tetraphyllidea*. Scolex with four bothridia (lappet-like outgrowths); vitellaria located in lateral margins of proglottids; genital pores lateral. Parasites of elasmobranchs (e.g. *Anthobothrium* sp.).

Order 2. *Lecanicephaloidea*.\* Reproductive system similar to Tetraphyllidea, but scolex divided into an upper disc-like or globular part and a lower collar-like part bearing four suckers. Mainly parasites of elasmobranchs (e.g. *Lecanicephalum* sp.).

Order 3. *Proteocephaloidea*. Scolex with four cyclophyllidean-like suckers and sometimes a fifth terminal one; vitellaria located in lateral margins; genital pores lateral. Mainly parasites of cold-blooded vertebrates (e.g. *Proteocephalus* sp.).

Order 4. *Diphyllidea*.\* Two bothridia each sometimes divided into two by a median longitudinal ridge. Large rostellum armed with a dorsal and ventral group of large hooks. Cephalic peduncle bears longitudinal rows of T-shaped hooks. Genital pore median. In elasmobranchs. Only a single genus: *Echinobothrium*.

Order 5. *Trypanorhyncha*. (Tetrahynchoidea.) Scolex with four spiny eversible proboscoides and two or four bothridia: vitellaria in continuous sleeve-like distribution. Parasites of elasmobranchs (e.g. *Grillotia erinaceus*).

Order 6. *Pseudophyllidea*. Scolex with two elongated shallow bothria; one dorsal and one ventral, segmented or unsegmented (Caryophyllacidae). Genital pore lateral or median. Vitellaria lateral or extending across proglottid encircling other organs. Parasites of teleosts and land vertebrates (e.g. *Diphyllbothrium latum*).

Order 7. *Nippotaeniidea*.\* Scolex bears a single apical acetabulum. Parasites of freshwater fish. Only a single genus: *Nippotaenia*.

Order 8. *Cyclophyllidea* (*Taenioidea*). Scolex with four acetabula; uterine pores lacking; a single compact vitellarium posterior to the ovary. Mainly parasites of birds and mammals (e.g. *Hymenolepis diminuta*).

Order 9. *Aporidea*.<sup>\*</sup> Rare forms lacking sex ducts or genital openings; ovary probably germovitellaria. Parasites of swans (e.g. *Nematoparataenia* sp.).

## 20.2 General Characteristics

### 20.21 Morphology

*External characters.* Typically a cestode is divided into a *scolex*, bearing attachment organs, followed by a short unsegmented region, the *neck*, succeeded by a chain of *proglottids* termed the *strobila*. The presence of a uterine pore usually defines the ventral surface, but external differentiation of surfaces may be difficult. Using internal features, the surface in proximity to the female system is defined as ventral.

The organs of attachment which occur on the scolex are of three types (Fig. 88): *bothria* (typical of the Pseudophyllidea) are long, narrow grooves of weak muscularity. In life, a bothrium may become extremely flattened to form an efficient sucking organ.

*bothridia* (phyllidea) (typical of the Tetraphyllidea) are broad, leaf-like structures with thin, flexible margins. They may be extremely variable, very mobile, stalked or sessile.

*acetabula* (suckers) (typical of the Cyclophyllidea) are true sucking organs, similar in structure to the suckers of the digenetic trematodes.

The scolex may be additionally armed with hooks. In the taeniod scolex, a mobile cone or *rostellum*, usually armed and retractable, is present.

*Proglottids.* The majority of adult cestodes are divided into segments or proglottids which arise by a series of transverse constrictions, the most recently formed proglottid being the one nearest the scolex. The process of proglottid formation is not understood, the zone of proglottid proliferation is presumed to be in the narrow neck region, but there is no cytological evidence that excessive somatic mitosis occurs here. As new proglottids form, the strobila elongates so that in some forms enormous lengths are achieved. In many groups (typically in the Cyclophyllidea) the most posterior proglottids become ripe first and when fully mature consist mainly of a branched uterus packed with eggs. Such a segment is said to be *gravid*. Gravid segments are often shed into the intestine and pass out with the faeces. Cestodes which shed ripe proglottids in this manner are said to be *apolytic* and those which retain them throughout life are termed *anapolytic*. Other terms used are: *euapolytic*—segments detached when nearly gravid;

*hyperapolytic*—segments detached much earlier and have a free existence in gut of host; *pseudoapolytic*—eggs liberated through uterine pore, segments then detached in groups and degenerate.

*Histology.* The structure of the cestode external covering has given rise to some controversy, but it is generally agreed that the following five layers exist:

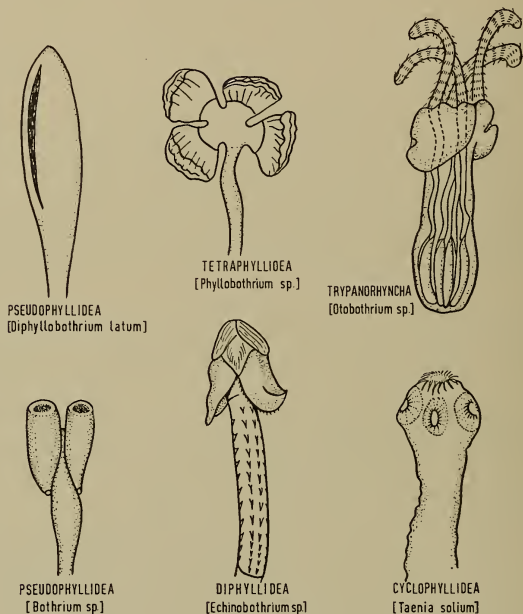


FIG. 88. Types of cestode scoleces (after various authors).

(a) *Cuticle.* The origin of the cuticle is obscure; it is probably secreted by sub-cuticular cells. Electron-microscope photographs (Kent, 1957) have shown that the cuticle has not a smooth regular surface, as it appears to have when viewed by the light microscope, but it is drawn into narrow elongated structures (cuticular 'villi') superficially resembling intestinal villi (Fig. 89). This seems clearly to be a device for increasing the absorptive surface of the worm.

(b) *Basement membrane*. Little is known regarding its structure; it is probably divided into two layers.

(c) *Sub-cuticular muscles*. These consist of two layers of myoblasts (muscle cells) with lateral fibre-like outgrowths (fibrillae). They are so arranged that one layer has its fibrillae running transversely to the long axis-forming 'transverse' or 'circular' muscles, while the fibrillae of the inner layer run longitudinally to form 'longitudinal' muscle bands. Longitudinal fibres predominate.

(d) *Neuromuscular cells*. These are multipolar cells with processes possibly connecting both to the sub-cuticular muscle cells and to the neurofibrillae from the nerve network.

(e) *Sub-cuticle*. This consists of numbers of sub-cuticular cells which occur beneath the sub-cuticular muscle layer. Each cell tapers at each end and opens out into branches whose upper poles penetrate the cuticle; these may be continuous with the cuticular 'villi' (Fig. 89).

*Parenchyma*. This fills all the spaces between the internal organs. As in trematodes it is a meshwork, probably syncytial, formed by anastomosis of mesenchymal cells. The spaces in the parenchymal meshwork are filled with a parenchymal fluid, often containing rich carbohydrate reserves in the form of glycogen.

*Nervous system*. This consists essentially of a pair of longitudinal trunks running the length of the strobila. Lateral accessory nerves are present and in taenioids, in addition there are a pair of dorsal and ventral nerves.

At least one ring commissure connects the longitudinal trunks in each proglottid and within the scolex a brain complex is formed, consisting basically of a pair of 'ganglia' united by a transverse median commissure and a ring commissure. The so-called 'ganglia' in the brain contain few nerve cells; these are mostly in the commissures. Special sense organs are lacking. Anterior nerves arise from the ganglia to supply the anterior region of the scolex; bothridial nerves and lateral nerves arise from the ganglion more posteriorly. Dorsal and ventral nerves may arise from the ring commissure and run both anteriorly and posteriorly. The complication of the nervous system in the scolex depends on that of the musculature.

*Excretory system*. Essentially a protonephridial system similar to that of Turbellaria with



FIG. 89. Submicroscopic structure of cuticle of *Hymenolepis diminuta*. Drawn from an electron micro-photograph by Dr. N. Kent. Note the cuticular 'villi'.

flame cells as excretory units. Typically, there are dorsal and ventral vessels on each side, but additional vessels may be present. The dorsal vessel is continuous with the ventral vessel of its own side forming a loop in the scolex. These two loops may be joined by a ring or a system of branches. In some cases (e.g. Pseudophyllidea) the excretory vessels may have a much more complicated arrangement, there being six longitudinal vessels plus a more superficial network.

## 20.22 Reproductive system

*General.* With the exception of the cyclophyllidean genus *Dioecocestus*, which is dioecious and dimorphic, cestodes are monoecious. The reproductive system follows the platyhelminth pattern. The female system in general resembles that of the digenetic trematodes, but in the Cyclophyllidea, the vitellaria are much reduced. The vagina of cestodes is homologous with Laurer's canal of the Digenea and with the vagina of Monogenea.

The reproductive system differentiates progressively from anterior to posterior end of the strobila. Most cestodes show a tendency towards protandry, the receptaculum seminis becoming filled with spermatozoa while the ovaries are maturing. The most posterior proglottids usually become laden with eggs and the remaining genitalia almost disappear; such proglottids are termed 'gravid'. In the Pseudophyllidea, however, large numbers of proglottids become mature at the same time. The cirrus sac and vagina usually open into a common genital atrium which opens externally by a single gonopore which may be lateral or central. In some species, the reproductive system is duplicated in each proglottid.

*Male.* Spermatogenesis follows the typical platyhelminth pattern. Testes may be in small dispersed groups or large well-formed bodies. Cirrus and cirrus sac are usually highly developed. Peculiarities arising are dealt with under the separate orders.

*Female.* Except in species with a double reproductive system, the ovary is single with two lobes, and its histological structure follows the typical platyhelminth pattern. The uterus takes on its characteristic shape only after sexual maturity is reached and it begins to fill with eggs. In tetracyllids and trypanorhynchids, it is characteristically a tube capable of distension to a sac which does not open by a pore to the outside when gravid.

In pseudophyllids, it usually takes the form of a much-coiled tube opening to the exterior by a median pore. In cyclophyllids, an external uterine opening is lacking and embryos are freed only by the disintegration of the shed proglottid. In these forms, the uterus develops extensive lateral diverticula, often of diagnostic value.



In some Davaineidae and a few Anoplocephalidae, a *paruterine organ* is present; this is a fibrous capsule surrounding the uterus or isolated pieces of uterus containing eggs.

*Eggs and egg-shell formation.* The prelarval development of very few cestodes has been worked out in detail. The segmented egg gives rise to an oval embryo, the *oncosphere*, or hexacanth larva, so-called on account of possessing three pairs of hooks at the posterior pole; it also possesses a pair of flame cells and some muscle fibres. In the taenioids, the embryo develops to the oncosphere stage in the uterus of mature proglottids but in the pseudophyllids embryonation only takes place when the eggs reach water. The larva hatching from the embryonated pseudophyllid egg is a *coracidium*, that is an oncosphere surrounded by a ciliated embryophore. Unlike the miracidium of digenetic trematodes, the coracidium does not enter the intermediate host actively but must be eaten by it. Consequently, the cilia are not indispensable and some coracidia are without them.

On the basis of the distribution of the vitellaria, the main orders of cestodes may be divided into two groups which form their eggs in different ways (Smyth and Clegg, 1959).

*Group I.* Cestodes, excepting Cyclophyllidea; with extensive vitellaria (Fig. 90). All the cestodes in this group lay their eggs in water and the first larval form passes into an aquatic intermediate host. Only the pseudophyllidean egg is well known and in some cases has a thick operculate capsule (= shell) (Fig. 90) like that of digenetic trematodes (Fig. 53).

The capsule is composed of sclerotin in three species investigated and the process of capsule formation is almost identical with that in trematodes (p. 144), the bulk of the egg-shell material being secreted by the cells of the vitellaria. As the vitelline cells mature, they increase in size and spherical globules appear in the cytoplasm. These globules are the shell precursors and give characteristic histochemical reactions for quinone-tanned proteins: phenolase, polyphenols and proteins (Smyth, 1957).

In the Tetraphyllidea and the Proteocephaloidea, the egg-capsule is thin and the eggs mature while still within the uterus and are ready to hatch or to be eaten whole on reaching water. It is possible that the thickness of the capsule may be closely related to the site where the egg matures, either in the water or in the uterus. On this view, it is considered that an egg which matures in the uterus does not need the protection of the thick capsule necessary for eggs which embryonated in water. This generalisation is based only on limited examples and may not hold when further species are investigated.

*Group II.* Cyclophyllidea; with a small, compact vitelline gland, which may be lacking in some cases (Fig. 91). In this group the eggs do not require an aquatic stage in the life cycle and always mature within the uterus which develops lateral branches.

Knowledge of the structure and development of the cyclophyllidean egg is fragmentary, the egg in the family Taeniidae being most investigated. The taenioid egg has a

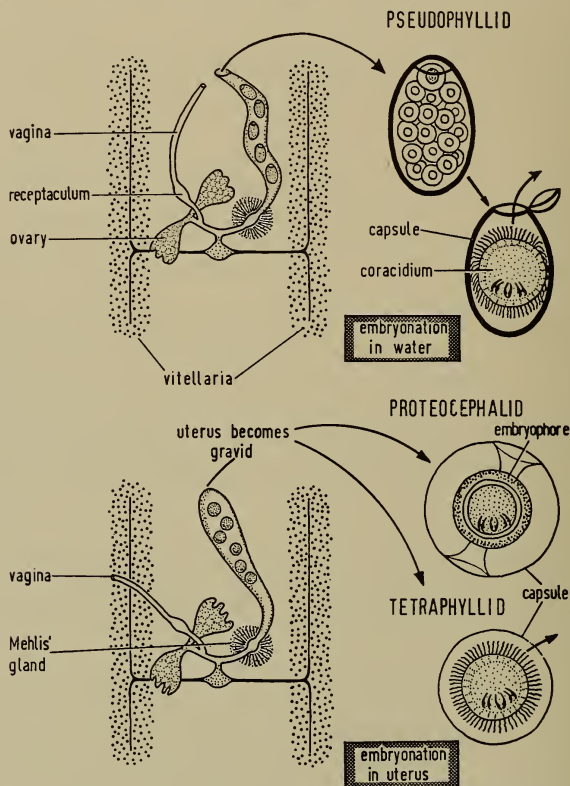


FIG. 90. Types of eggs formed in cestodes (Group I, see text) with extensive vitellaria (after Smyth and Clegg, 1959).

thin capsule which is separated from a greatly thickened *embryophore* only by a narrow granular layer (Fig. 91). In some species (e.g. *T. saginata*) the capsule is delicate and usually absent in mature ova, so that what appears to be the 'shell' is, in reality, the

thickened embryophore. The embryophore is made up of hexagonal columns cemented together, the cement substance being digested by the action of pepsin (in *T. saginata*) or

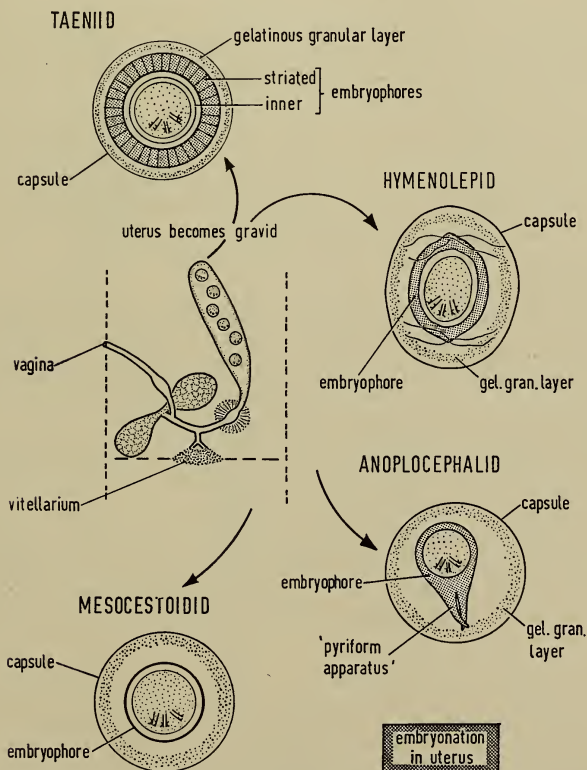


FIG. 91. Types of eggs formed in cyclophyllidean cestodes (after Smyth and Clegg, 1959).

trypsin (in *T. pisiformis*) (Silverman, 1954). The released oncosphere is still enclosed in a lipoidal oncospherical membrane and requires further stimulation (by pancreatin and bile salts) before it can break out (see p. 258).

In other families, the thickness of the capsule and embryophore varies, as does the

ease with which hatching occurs. Oncospheres of some species can hatch in distilled water, but those of others require enzyme treatment.

In the Hymenolepididae, the capsule is thick and remains around the mature egg (p. 231), and the embryophore, which is not as thick as in the Taeniidae, has filaments on either pole. In the Anoplocephalidae, the egg is similar but the embryophore has a pair of horns (forming a 'pyriform apparatus') at one pole. Not all cyclophyllidean eggs have a thickened embryophore; in the Mesocestoididae, the embryophore is a thin cellular membrane separated from the capsule by a granular layer.

*Insemination.* Whether self-fertilisation within the same proglottid or cross-fertilisation between different proglottids occurs, copulatory processes are only made possible in cestodes by the compression of the strobila against the intestinal wall. Thus fertilised eggs are only produced *in vitro* if the strobila is compressed during maturation; this is normally achieved by an artificial gut of cellulose tubing (p. 422).

### 20.23 Life cycles

With the exception of *Hymenolepis nana*, whose eggs can develop directly within the rat intestine (p. 256), cestodes require an intermediate host for completion of the life cycle. A wide range of homoiothermic and poikilothermic vertebrate and invertebrate intermediate hosts are utilised, the cyclophyllidean cestodes using mainly terrestrial species and the remaining orders mainly aquatic species.

In all cases, infection of the intermediate host takes place orally, the ingested larvae penetrating the gut by means of its hooks and being carried to its site of development. The following types of larvae are recognised (Fig. 92):

*Metacestode.* This term is used to describe a stage, or a succession of stages, passed within the intermediate host; it is one which does not normally become sexually mature. Neotenic larvae occur in some species (see p. 247). A number of types of metacestodes are found and these may be grouped according to whether or not they show budding (Fig. 92).

(a) those which do not show budding:

- (i) *proceroid*: a small spindle-shaped larva with a solid body with posterior hooks; the first larval stage of pseudophyllideans or trypanorhynchids (e.g. larva of *Schistocephalus solidus* in copepods).
- (ii) *plerocercoid*: the second larval stage of Tetracyllidae, Pseudophyllidae and Trypanorhyncha; also (more rarely) in taenioids (e.g. *Paruterina* sp.). A solid larva possessing an adult scolex but lacking the embryonic hooks of the proceroid from which it was developed (e.g. larva of *Diphyllobothrium* sp.).
- (iii) *cysticercoid*: a larva consisting of an anterior vesicle containing the scolex, which is not invaginated, and a tail-like posterior region containing the larval

hooks (which may persist for some time) (e.g. larva of *Hymenolepis diminuta* in *Tenebrio molitor*).

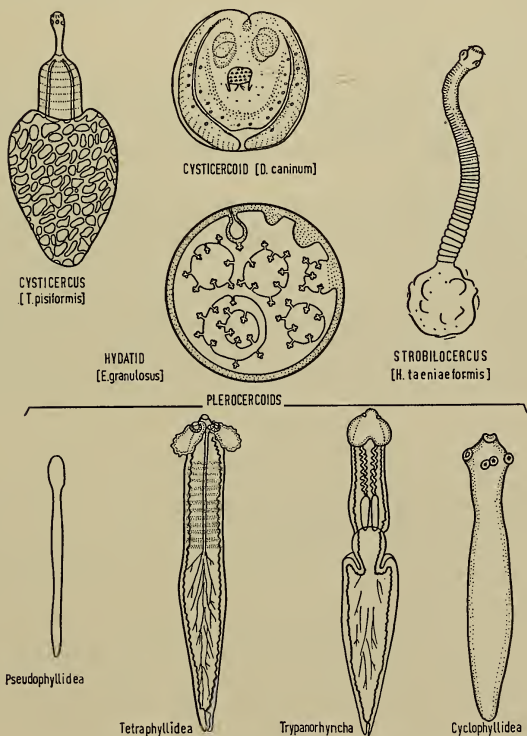


FIG. 92. Types of cestode larvae (after various authors).

- (iv) *cercocystis*: a cysticercoid with a tail-like appendage from the bladder (e.g. larva of *Hymenolepis diminuta* in the larva of grain beetle *Tenebrio molitor*).
- (v) *cryptocystis*: a cysticercoid in which the tail is present early in development only (e.g. larva of *Dipylidium caninum* in the flea *Ctenocephalus canis*).
- (vi) *cysticercus*: a bladder enclosing a single scolex retracted and invaginated within itself. Usually in vertebrates (e.g. larva of *Taenia pisiformis* in rabbits).

- (vii) *strobilocercus*: scolex usually not invaginated and connected by a long, solid, segmented strobila to a small bladder (e.g. larva of *Hydatigera taeniaeformis* in rodents).
- (b) those which do show budding:
- (i) external budding:  
*Urocystis* sp.: undergoes early budding before cysticercus is formed.  
*Urocystidium* sp.: undergoes late budding from young strobila (e.g. from *strobilocercus*; sometimes called a staphylocystis).\*
- (ii) internal budding:  
*monocercus*,\* *polycercus*:\* of cysticercoid type; scolex or scolices are budded from wall and become free in the bladder (e.g. larvae of *Paricterotaenia nilotica* in earthworm).  
*coenurus*: of cysticercus type; groups of scolices are budded from wall of bladder and remain connected to wall (e.g. larva of *Taenia multiceps*).  
*hydatid*: of cysticercus type; scolices do not develop in walls of bladder but within vesicles termed 'brood capsules' (e.g. larva of *Echinococcus granulosus*).

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\* rare.



## CHAPTER XXI

### EUCESTODA: 'MINOR' ORDERS

*Order 1. Tetraphyllidea.* The tetraphyllids are mostly small cestodes, exclusively parasitic in elasmobranchs. Their life cycles are not completely known but copepods, fish, ctenophores and other marine organisms have been found to contain larvae which are undoubtedly tetraphyllid proceroids or plerocercoids as adjudged by the pattern of the scolex.

The scolex is characterised by the possession of four bothridia (Fig. 88) which may vary in size and shape. The proglottids possess a single reproductive system with a lateral genital opening. The cirrus is usually armed with hooks, hairs or spines. The vitellaria are lateral (e.g. *Phyllobothrium dohrni* from the intestine of the skate).

*Order 2. Lecanicephaloidea.* A small order, exclusively parasites of elasmobranchs. The scolex is divided into two portions—an upper disc-like or globular part sometimes bearing tentacles, and a lower collar-like part, bearing suckers like those of the cyclophyllideans. Reproductive system practically identical with tetraphyllids. Life cycle largely unknown (e.g. *Lecanicephalum* sp. from spiral valve of elasmobranchs).

*Order 3. Proteocephaloidea.* The proteocephalids combine a tetraphyllid type of reproductive system with a somewhat cyclophyllidean type of scolex. They are typically parasites of fresh-water cold-blooded vertebrates with a few exceptions in elasmobranchs.

The principle genus is *Proteocephalus* (Fig. 93) with numerous species in fresh-water fish, amphibians and reptiles. A species commonly available in Great Britain is *Proteocephalus fillicollis* (syn. *Ichthyotaenia filicollis*) in the sticklebacks, *Gasterosteus aculeatus* and *Pygosteus pungitius*. It is a small species, seldom longer than 35 mm, producing large numbers of eggs with quinone-tanned capsules. These embryonate in water and hatch out typical coracidia. If a coracidium is eaten by a copepod, typically *Cyclops strenuus*, it penetrates the haemocoel and develops into a proceroid larva which later becomes a plerocercoid larva. This life cycle is essentially an abbreviated form of the pseudophyllid life cycle which is discussed in detail later (p. 241). Proteocephalid life cycles have been little studied and nothing is known regarding the growth rates nor the physiology of the various stages.

*Order 4. Diphyllidea.* A little-studied order containing only the single genus *Echinobothrium* (Fig. 88). Larval stages occur in marine molluscs and crustaceans. Main morphological features given on p. 224.

*Order 5. Trypanorhyncha.* Easily distinguished from other orders by the presence of four spiny *proboscoides* which lie in sheaths and can be everted from the scolex. The most complete work on tetrarhynchs is that of Dolfuss (1942). Rees (1944, 1950) and Johnstone (1911) have made detailed anatomical studies of several species.

*Type Example: Grillotia erinaceus*

definitive host:	<i>Raia</i> spp.
location:	intestine
first intermediate host:	<i>Acartia longiremis</i> , <i>Pseudocalanus elongatus</i> , <i>Paracalanus parvis</i> , <i>Temora longicornis</i>
second intermediate host:	<i>Gadus</i> spp., <i>Trigla</i> spp., <i>Lophius piscatorius</i> and other marine teleosts
distribution:	widespread

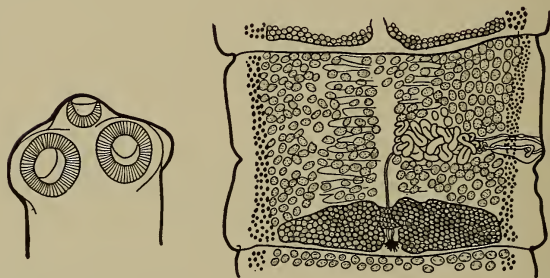


FIG. 93. *Proteocephalus osculatus* (order Proteocephaloidea)—morphology of scolex and proglottid (after Nybelin, 1942).

This parasite is most widely known through its larva which occurs encysted in many marine fish. It is among the commoner helminth parasites of fishes from the Irish Sea. It is rare for the whiting or the gurnard to be free from infection.

*Morphology.* The morphology of the adult from ray is shown in Fig. 95. Specimens rarely exceed 60 mm in length. The posterior proglottids are continually becoming detached and are often found free; they show no exceptional morphological features. Each *proboscis* is a tube lined internally with a variety of different-sized hooks so that when everted the spiny side is outwards. It is contained in a tube or *proboscis sheath* which terminates in an oval muscular mass, the *proboscis bulb*. The whole appara-

tus is filled with a fluid and the proboscoides are everted by contraction of the muscular bulbs. A retractor muscle effects the withdrawal of the proboscis into the sheath.

**Life cycle.** The life cycle resembles that of the Pseudophyllidea (Ruszkowski, 1934). The eggs embryonate in sea water in eight days (temperature not stated) and hatch out typical ciliated coracidia. When taken in by copepods of the species listed above, these develop into proceroids closely resembling those of pseudophyllids. When infected copepods are ingested the larvae are freed and bore through the wall of the alimentary canal to encyst beneath the serous layer or the peritoneum of the body wall (Figs. 94, 95).

Numerous other species occur in sharks and rays, but their life cycles have not been worked out. Advanced larvae, mainly plerocercoids but sometimes cysticeroids, have

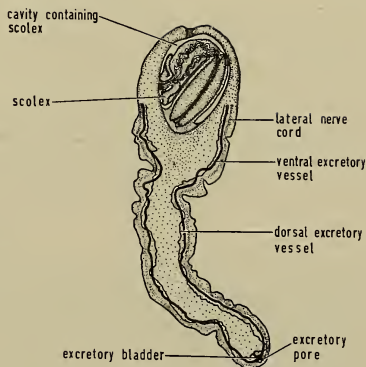


FIG. 94. Plerocercoid larva of *Grillotia heptanchi* from the hake (*Merluccius merluccius*)—note that the scolex is invaginated in the anterior part of the body of the larva (after Rees, 1950).

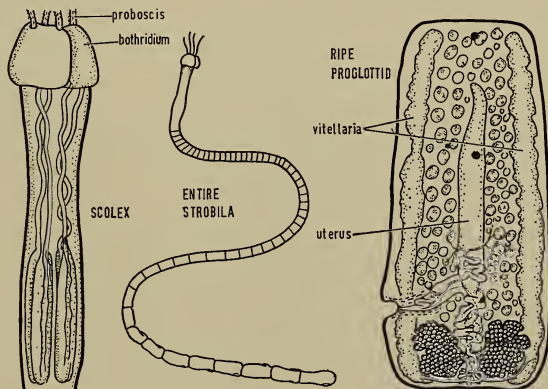


FIG. 95. *Grillotia erinaceus* (order Trypanorhyncha)—plerocercoid removed from cyst (from body cavity of *Gadus* sp.) and adult strobila and proglottid (from intestine of ray) (after Johnstone, 1912).

been recorded from numerous marine animals (fish, gastropods, lammellibranchs, medusae), but proceroids have not been described. Rees (1941) has given a detailed description of the anatomy of a plerocercoid.

*Order 7. Nippotaeniidea.\** This order contains only the single genus *Nippotaenia*—small cestodes from Japanese fish. They have no proper scolex but only a single acetabulum.

*Order 9. Aporidea.\** This order contains rare monozoic forms, first described in swans. The ovary appears to be a germovitellaria and the testes form a mass running most of the body length. There appear to be no sex ducts.

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\* For convenience these orders are included here.

## CHAPTER XXII

### EUCESTODA: PSEUDOPHYLLIDEA

Members of this order, commonly referred to as pseudophyllids, are chiefly parasites of fish-eating mammals, birds and fish other than elasmobranchs. In typical forms, the scolex is characterised by two shallow elongated bothria situated one dorsally and one ventrally (Fig. 88), and proglottids are flattened dorso-ventrally. The median genital pores are dorsal or ventral in position. This order contains a number of species of value in experimental work, or of economic importance. Most of these belong to the family Diphylobothriidae.

#### 22.1 Genus *Diphylobothrium*

##### 22.11 Type Example: *Diphylobothrium dendriticum*

definitive hosts:	natural: herring gull, common gull, greater black-headed gull
laboratory:	rat, kitten
location:	small intestine
first intermediate host:	fresh-water copepods: <i>Cyclops</i> spp., <i>Diaptomus</i> spp.
second intermediate host:	various fresh-water teleosts, especially <i>Gasterosteus aculeatus</i> (stickleback), <i>Salmo trutta</i> (trout-paratenic host)
distribution:	widespread, especially in lake districts

This parasite has a structure and life cycle closely paralleling that of the *Diphylobothrium latum* (of man), but it is more readily available and has the advantage of developing in the laboratory rat (Kuhlow, 1953; Archer and Hopkins, 1958).

**Morphology.** The morphology of a diphylobothriid proglottid (Fig. 96), is too well-known to require detailed description. Both on morphological and histochemical grounds the genitalia bear a striking resemblance to that of the Trematoda (Fig. 97) with which it may have affinities.

There are three genital openings: the vagina, the uterus and the cirrus respectively.

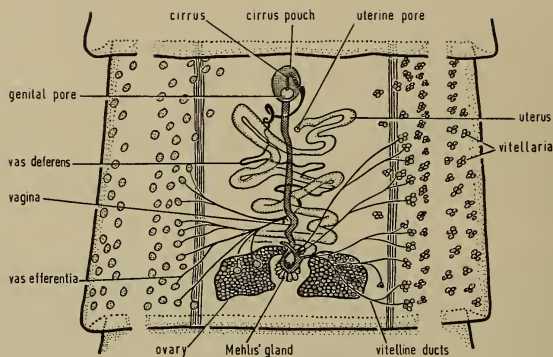


FIG. 96. Morphology of proglottid to illustrate the general features of diphyllid organisation (after Brown, 1950).

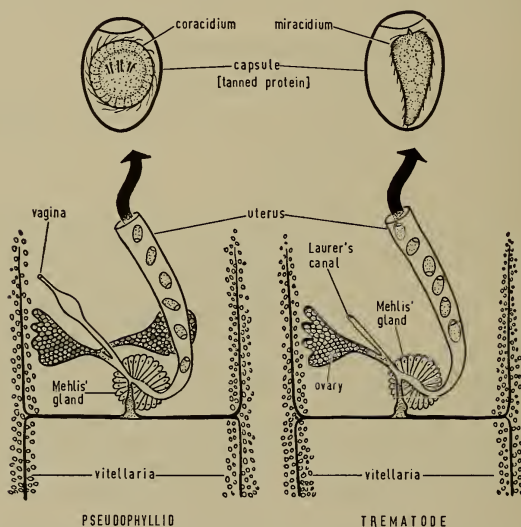


FIG. 97. Comparison of the genitalia in trematodes and pseudophyllid cestodes (original).



The cirrus and vaginal openings are in a common atrium in some species. The vagina runs posteriorly from its opening and joins the oviduct before entering an ootype surrounded with the cells of Mehlis' gland. The secretion of these glands, like that of trematodes, gives a strong Periodic Acid Schiff reaction, but its function is unknown. The vitellaria are scattered throughout lateral fields and their ducts join to form a short median duct which swells to form a vitelline reservoir before entering the ootype.

There is a bilobed ovary leading by an oviduct into the ootype. The uterus consists of a series of coiled tubes increasing in size posteriorly. The male system follows a typical pattern with testes capsules widespread and the cirrus and cirrus sac well developed. The egg shells are quinone-tanned protein and are formed from the vitelline globules; the vitellaria give strong histochemical reactions for proteins, phenols and phenolase, as in trematodes (p. 143).

#### *Life cycle* (Fig. 98).

A large proportion of proglottids, which are pseudoapolytic (p. 226), mature at the same time, and quantities of eggs appear in the faeces. For obvious ecological reasons, faeces from fish-eating birds have a good chance of reaching water. At 15° C., eggs embryonate in thirty days; at 25° C., in eight days. Eggs are readily embryonated in the laboratory on cellophane discs placed in watch glasses enclosed in petri dishes.

Embryonated eggs hatch only on exposure to light. Presumably the mechanism here is the same as in the case of the eggs of *Fasciola*, in which there is evidence that a light-released enzyme attacks the opercular seal (p. 145). The retardation of hatching by darkness is useful in laboratory procedures as release of coracidia from embryonated eggs can be withheld until suitable cultures of *Cyclops* are available. The hatched larva is a *coracidium*, essentially a hexacanth embryo enclosed in a ciliated embryophore. It swims actively with a ciliate-like motion and exhibits a tendency towards negative geotropism. It contains little food reserves and dies if not eaten by a copepod within about twelve hours.

*Infection of copepod.* A number of copepods can act as intermediate hosts: *Cyclops strenuus*, *Diaptomus gracilis*, *D. graciloides*, *D. vulgaris* (Kuhlow, 1953). The copepodid stages are more easily infected, the mature copepods being often refractory to infection. *D. gracilis* and *D. graciloides* are the most susceptible to infection. When ingested by a copepod, the embryophore is shed and the contained hexacanth bores its way rapidly through the intestine into the haemocoel. Little is known of the properties of the copepod haemocoel.

The rate of growth of the procercoid larva which now develops has not been

determined for this species, but it is unlikely to be much different from that of another diphyllbothriid, *Schistocephalus solidus* (Fig. 104).

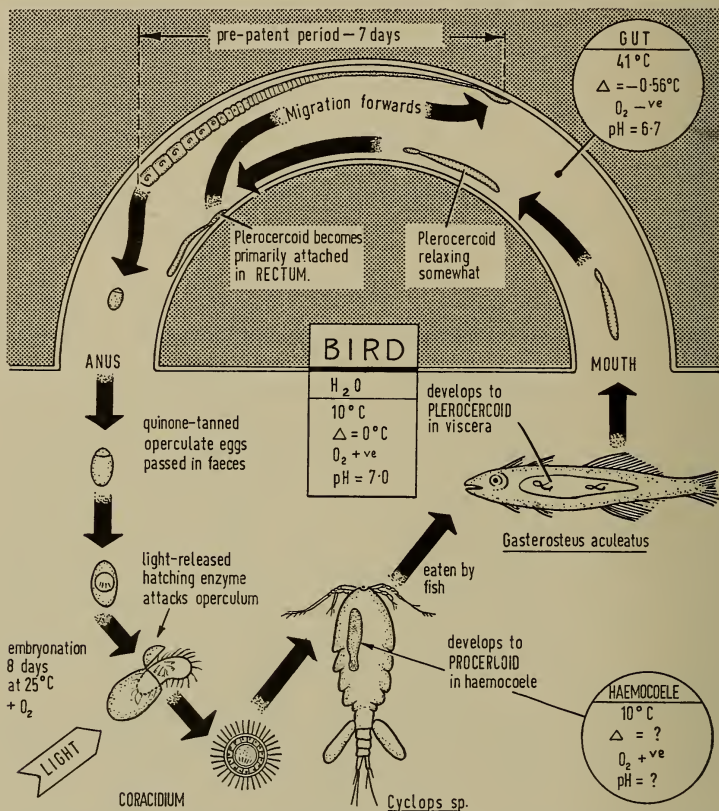


FIG. 98. *Diphyllbothrium dendriticum*—life cycle and some physiological factors relating to it (original).

A proceroid reaches an infective stage when the hooks become separated into a constricted posterior region termed a *cercomer* (Fig. 99).

*Infection of fish.* When a *Cyclops* containing a procercoid in the infective stage is eaten by a stickleback, it develops into the final larval stage, the plerocercoid (Fig. 99). It is a white, opaque, elongate structure, with a well-differentiated scolex. The latter is usually contracted and partly invaginated, and its true nature is not revealed until stimulated to expand and evaginate by immersion in warm saline. Plerocercoids encyst in various parts of the body but are occasionally found in the body cavity. They show

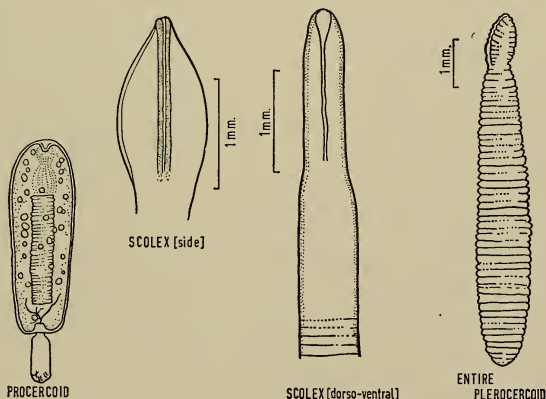


FIG. 99. *Diphyllobothrium dendriticum*—anatomy of plerocercoid, procercoid and adult scolex (after Kuhlöw, 1953).

a marked predilection for the viscera in the anterior part of the body cavity, especially the liver and stomach wall, but rarely occur in the gonads.

It has been shown in some species that if infected fish are eaten by larger fish, the plerocercoids penetrate the gut and become re-encapsulated in its vicinity. Hundreds of plerocercoids may thus accumulate in this second fish which is known as a *paratenic host*. If the second fish is a large one, such as a pike, not normally eaten by a bird, the plerocercoids it accumulates will have no chance of developing to maturity and are thus withdrawn from the life cycle.

In the case of *D. dendriticum*, it is not known whether a trout acts as a paratenic host or not but this is likely. Autopsy of stomachs reveal that trout apparently do not feed on copepods, but almost exclusively on sticklebacks if these are available.

*Infection of definitive host.* The normal definitive hosts of *D. dendriticum* are gulls, *Larus*

*canus*, *L. argentatus*, *L. ridibundus* and others, but development has not been obtained in other common fish-eating birds which frequent reservoirs, such as *Phalacrocorax carbo* (cormorant), *Phalacrocorax graculus* (shag), *Ardea cinerea* (heron).

Development can take place in a number of laboratory animals (Table 32). In mammalian hosts, development may be abnormal and a proglottid may show duplication or even triplication of its genitalia. The pattern of behaviour and development may, therefore, vary considerably in different hosts, a phenomenon well recognised in other helminths (pp. 185, 195, 255).

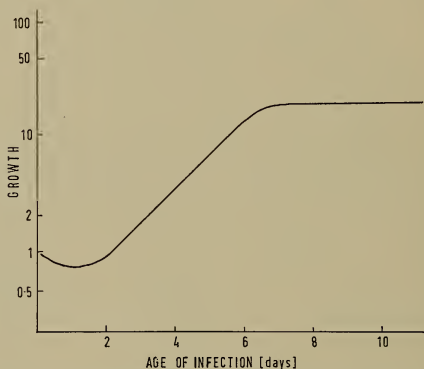


FIG. 100. Rate of growth of *Diphyllobothrium* sp. (probably *D. dendriticum*) in small intestine of rat; growth plotted on log scale as multiple of fresh weight (redrawn from Archer and Hopkins, 1958, omitting scatter points).

*Growth in the definitive host.* The rate of growth in the rat has been studied by Archer and Hopkins (1958). If rats are autopsied 12–24 hrs after feeding with plerocercoids, it is found that the latter all occur in the region of the large intestine. Yet if autopsied after 3–7 days, the strobilae are found to be established in the duodenum. This clearly points to a remarkable process of forward migration from the rectum to the duodenum.

The rate of growth is shown in Fig. 100, and the morphological characteristics of the various phases are given in Table 33. There is an initial lag during the first two days during which time no growth occurs. Thereafter the strobila grows rapidly, reaching maturity between the sixth and seventh days. Maturation is slightly more rapid in the gull (41° C.—6 days) than in the rat (38.8° C.—7 days), an effect directly attributable to differences in temperature. Archer and Hopkins (1958) have shown that the weight of

TABLE 32  
DEVELOPMENT OF *DIPHYLLOBOTHRIUM DENDRITICUM* IN  
EXPERIMENTAL HOSTS  
(data from Kuhlowl, 1953)

Species	No. of feeding experiments	No. of successful infections	Per cent infections
<i>Anas boschas domestica</i> . . .	2	0	0
<i>Ardea cinerea</i> . . .	3	0	0
<i>Columba domestica</i> . . .	1	0	0
<i>Larus argentatus</i> . . .	5	3	60
<i>Larus canus</i> . . .	13	10	77
<i>Larus ridibundus</i> . . .	59	24	40
<i>Serinus canariensis</i> . . .	1	0	0
<i>Sterna hirundo</i> . . .	2	1	50
<i>Turdus merula</i> . . .	1	0	0
<i>Canis familiaris</i> . . .	2	1	50
<i>Felis domestica</i> . . .	3	2	66
<i>Cavia cobaya</i> . . .	1	0	0
<i>Mus musculus</i> . . .	6	2	33
<i>Rattus rattus</i> . . .	3	1	33
<i>Homo sapiens</i> . . .	3	0	0

the adult maturing (Wa) in rats on the seventh day is directly proportional to the weight of the plerocercoid (Wp) initially ingested. Thus

$$W_a = W_p K.$$

About 70 per cent of the strobila matures on the seventh day, indicating that the proglottids in nearly three-quarters of the body are of the same age. Growth in this species *in vitro* is discussed further on p. 423.

TABLE 33  
DEVELOPMENTAL PHASES OF *DIPHYLLOBOTHRIUM DENDRITICUM* DURING  
MATURATION IN THE INTESTINE OF THE RAT  
Body temperature 38.8° C. (from Bell and Smyth, 1958)

Phase	Incubation time (days)	Characteristics
(1) Cell multiplication . . .	0-1	numerous mitoses
(2) Segmentation . . .	1-2	appearance of proglottids
(3) Organogeny . . .	2-3	appearance of uterus and testes primordia
(4) Early gametogeny . . .	4-5	appearance of early stages in spermatogenesis
(5) Late gametogeny . . .	5-6	appearance of mature spermatozoa
(6) Egg-shell formation . . .	6-7	appearance of egg-shell precursors in 'vitelline' cells
(7) Oviposition . . .	7-8	appearance of fully-formed egg

**22.12 *Diphyllbothrium latum*—the human 'broad' tapeworm**

*General account.* The largest tapeworm found in man. It has a length of 10–40 feet and a width of 10–20 mm. It occurs especially in countries addicted to eating fish in an uncooked or partly cooked (i.e. smoked) condition. The term 'broad' relates to the fact that the proglottids are usually wider than they are long.

*Distribution.* It occurs especially in central Europe, but has a world-wide distribution. It also occurs in parts of Canada and the United States, and in Siberia, Palestine, Japan, Central Africa, Chile and Australia. Infected immigrants can readily introduce it into any country provided suitable copepod and fish hosts occur.

*Morphology.* The general pattern of the morphology (Fig. 96) does not differ significantly from that of *D. dendriticum* except in size and number of proglottids. The worm is greyish-yellow to brown in colour and may have 3,000–4,000 proglottids.

*Life cycle.* The life cycle differs from *D. dendriticum* only in detail, the main point of difference being that the plerocercoids do not encyst but lie usually in the muscles and rarely in the body cavity or viscera of the fish intermediate host. Many species of fish have been incriminated and in some countries (e.g. parts of Russia) practically all food fish are infected. The larger carnivorous fish are paratenic hosts, becoming infected by eating smaller plankton-eating fish. The larger fish involved are different in different countries: in Europe—trout, perch and turbot; in U.S. and Canada—pike and walleyes; in the Far East and Chile—trout; in Africa—the barbel.

Many carnivorous animals are natural hosts and act as important reservoirs for human infections; species of the dog and cat families are especially incriminated.

*Growth rate.* The prepatent period in the dog is twenty-one days, precisely three times longer than *D. dendriticum* (Wardle and Green, 1941). A mature proglottid of *D. latum* is approximately three times the weight of *D. dendriticum* (estimated from the figures of Archer and Hopkins, 1958; Wardle and Green, 1941), so that considerably more tissue synthesis is required before maturation can take place.

**22.13 'Sparganum' infections—fate of plerocercoids in non-alimentary habitats**

When plerocercoids of *D. dendriticum* are transferred artificially to a site in a warm-blooded host, such as the body cavity, in which little soluble growth materials are available, maturation does not take place, although larvae may survive (without differentiation) for lengthy periods.

In the majority of species of the family Diphyllbothriidae, the chances of a plerocercoid from a fish muscle having an opportunity of entering the body cavity or tissue



sites of the homoiothermic host are infinitesimal. One species of the genus *Spirometra*, closely related to *Diphyllbothrium*, however, has such an opportunity. Its plerocercoids occur first in tadpoles and later in frogs, reptiles and mammals in which they become fully developed plerocercoids. Natives in Indo-China, China and Japan especially use fresh-split frogs to poultice wounds and sore eyes, and so active plerocercoids have ready access to tissue sites. Within these sites they may grow to considerable lengths (3-4 in.) without undergoing further differentiation. These well-developed plerocercoids are known as *spargana* and they can develop in a wide range of hosts. In some cases, if spargana are eaten by favourable hosts, especially cats and dogs, they develop into sexually mature intestinal tapeworms of the species *Spirometra mansonioides*. In unfavourable hosts, the spargana merely re-establish themselves in the tissues and continue to grow in size without differentiation.

## 22.2 Pseudophyllidea with progenetic\* proceroids

Although the majority of plerocercoids do not reach a high degree of morphological development while within the intermediate host, there are some outstanding exceptions. The most remarkable of these are the genera *Archigetes* and *Caryophyllaeus* in oligochaetes and fish respectively.

'*Archigetes*' *sieboldi* (Fig. 101). The adult of this species occurs in the intestine of fresh-water teleosts and the proceroid in the coelom of fresh-water oligochaetes, chiefly Tubificidae. The oligochaete coelom is evidently an environment of a high nutritional level, for the proceroid grows and develops reproductive organs which finally become mature. Maturation will also take place in various fish which must thus be regarded as the true definitive hosts. This remarkable case of progenesis has been known for many years and the name *Archigetes* was given to the progenetic larva (believed to be a plerocercoid) before the adult, *Biacetabulum sieboldi*, was identified (Szidat, 1937). The anatomy of *Archigetes* is shown in Fig. 101. The egg capsules do not hatch until eaten by the oligochaetes.

*Caryophyllaeus* sp. (Fig. 101). Proceroids of this species inhabit the coelom of fresh-water oligochaetes and although the reproductive organs reach the organogeny stage of development, complete sexual maturation is not reached until taken into a definitive fish host.

\*The terms *progenesis* and *neoteny* are almost synonymous. Maturation of the gonads in a larval animal is known as *neoteny*. Advanced development of genitalia in a larva (without actual maturation) is known as *progenesis*. An advanced progenetic condition clearly becomes *neoteny*. *Archigetes* is strictly speaking a *neotenic* larva but the terms *neotenic* and *progenetic* are rather loosely used. See also pp. 151, 158.

### 22.3 Pseudophyllidea with progenetic plerocercoids

This group contains only the genera *Schistocephalus* and *Ligula* whose plerocercoids, which occur in fresh-water fish, have been widely used for experimental work *in vitro* (p. 421).

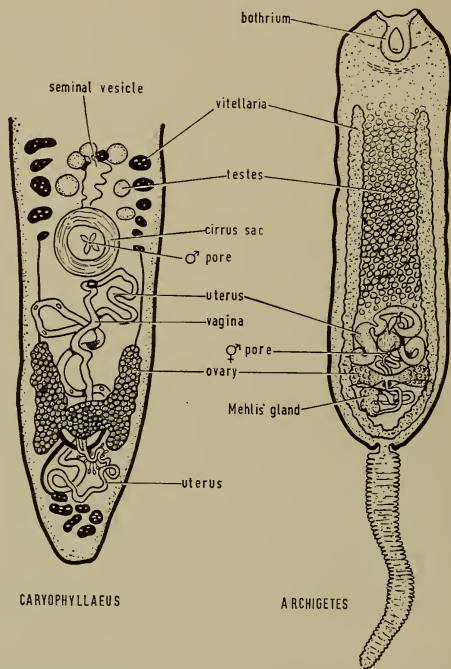


FIG. 101. '*Archigetes*' *sieboldi* from the body cavity of fresh-water oligochaetes. *Caryophyllaeus* sp. from the gut of fresh-water teleosts (after Wisniewski, 1930; Hunter, 1927).

#### 22.31 *Schistocephalus solidus*

**Occurrence.** The adults occur in a wide range of bird hosts, but due to the rapid maturation and short life in the bird, may be rarely found on autopsy even in areas where fish infected with plerocercoids abound. The worm is non-specific in its choice of definitive

host and maturation will probably take place successfully in any bird. Pigeons are suitable laboratory hosts. The worm has also been reported from the otter and other fish-eating mammals and on theoretical grounds (see below) could mature in the intestine of any warm-blooded host and only physical or ecological barriers probably prevent it doing so.

The wide host spectrum is related to the progenetic nature of the plerocercoid (Fig. 102) which has sufficient food reserves (Table 35) to enable it to mature without the assistance of exogenous food material; a temperature of 35–40° C. and suitable physico-chemical conditions suffice to assure maturation *in vitro* (p. 422).

**Morphology.** The adult (Fig. 103) is a lanceolate-shaped worm with a size range of 5–8 cm by 1 cm. Bothria on the scolex are represented by a short median groove which possesses no adhesive powers. The adult strobila is thus unable to hold on to the intestinal wall. The lack of bothria, then, can be co-related with the rapid rate of maturation (thirty-six hours), since a worm is unable to remain in the host intestine for longer than three days. That it can remain even so long in the gut is presumably due to an ability to brace itself by muscular action against the peristalsis and the intestinal current. It may be significant that this species has an additional band of circular muscles.

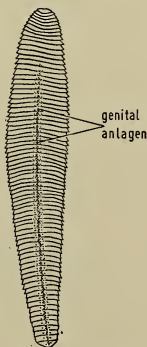


FIG. 102. Progenetic plerocercoid of *Schistocephalus solidus* from the body cavity of the stickleback, *Gasterosteus aculeatus* (original).

**Plerocercoid** (Fig. 103). The plerocercoid has the main features of the adult, division into proglottids and the presence of genitalia, but the latter reach only the organogeny state of development (see p. 419). The intermediate hosts in Europe are the three-spined stickleback, *Gasterosteus aculeatus*, and the ten-spined stickleback, *Pygosteus* sp. These are small fish seldom more than three inches long, and relative to their size, plerocercoids may become enormously developed, parasites sometimes weighing up to 90 per cent of the host tissue! Strangely enough, although their movements become more sluggish, fish remain relatively unaffected by the infection; maturation of the gonads may be retarded but never completely inhibited (compare *Ligula*, p. 252, whose plerocercoids produce complete parasitic castration in fish).



FIG. 103. *Schistocephalus solidus*. Morphology of adult—from specimens matured *in vitro* (see p. 422) (original).

Chemical analysis (Table 35) shows that a plerocercoid contains abundant carbohydrate reserves in the form of glycogen (making up some 50 per cent of the dried weight) localised mainly in the parenchyma and muscles. The majority of infected fish

contain only one to four large-sized plerocercoids, but up to ten medium-sized larvae may sometimes be found.

*Life cycle.* The life cycle (Fig. 105) follows the typical cycle already described for *D. dendriticum* with corresponding differences in times for maturation in the definitive hosts as already stressed. A convenient laboratory host is the common *Cyclops viridis* whose copepodid stages are readily infected, 30–40 proceroids being common in experimental infections. As with other pseudophyllids, adult copepods exhibit resistance to infection.

At summer temperatures (15–18° C.) development of the proceroid to the cercomer stage takes approximately twenty-five days. Fish are infected by ingesting

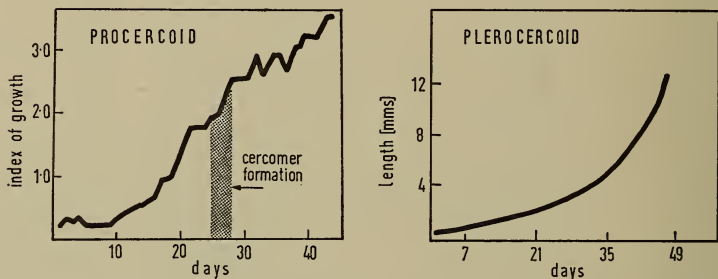


FIG. 104. *Schistocephalus solidus*—growth of proceroid in *Cyclops*, and plerocercoid in *Gasterosteus* at room temperatures (modified from Clarke, 1954).

infected copepods. The contained proceroid rapidly penetrates the fish gut and within two hours may be found in the body cavity. The growth of a plerocercoid is shown in Fig. 104. Segmentation commences when a larva is about 8–9 mm. The genitalia make their appearance in the middle proglottids first and develop anteriorly and posteriorly. As the worm becomes fully developed, plerocercoid genitalia appear in all the proglottids, with the exception of the twelve to thirteen most anterior ones which are sterile. These genitalia become organised to the early gametogeny stage of maturation, that is the main tubular genitalia appear and both ovaries and testes develop but neither spermatogenesis nor oogenesis proceed further (contrast *Bucephalopsis*, p. 158). As soon as proglottids develop, and even a single proglottid contains well-developed genitalia, a plerocercoid is infective. The smallest plerocercoid capable of reaching maturity is about 10–15 mg, but plerocercoids may grow to over 500 mg. The average size is

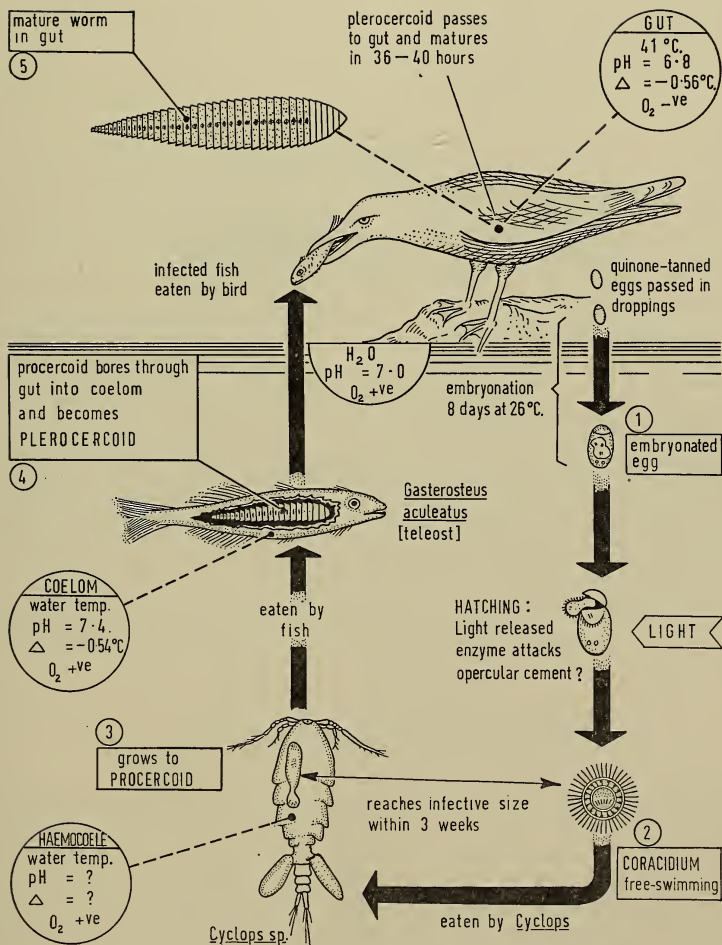


FIG. 105. Life cycle of *Schistocephalus solidus* and some physiological factors relating to it (from Smyth, 1959).

about 200 mg. The life cycle has been summarised by Hopkins and Smyth (1951), and Clarke (1954).

Provided suitable physico-chemical conditions are available, the only stimulus required to induce the genitalia to become mature is a rise of temperature to that of a

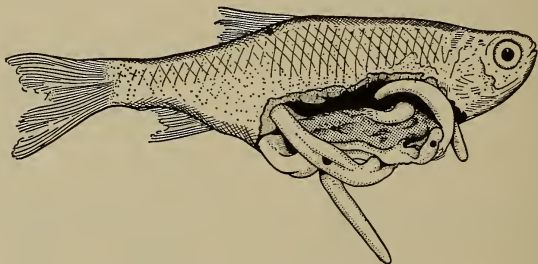


FIG. 106. Roach (*Leuciscus rutilus*) infected with plerocercoids of *Ligula intestinalis* (original).

bird (40° C.), a process which can be accomplished *in vitro* (Smyth, 1954; 1959, see p. 422). Maturation is rapid and thirty-six hours after the plerocercoid enters a bird gut, eggs may appear in the uterus. Normal maturation will not take place below 35° C.

### 22.32 *Ligula intestinalis*

This member of the family *Diphyllbothriidae* has a structure and life cycle closely resembling *Schistocephalus solidus*. The best-known form is the plerocercoid which has

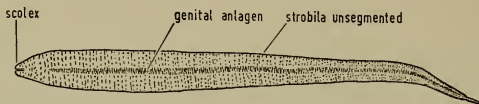


FIG. 107. Progenetic plerocercoid of *Ligula intestinalis* removed from body cavity of roach (original).

been reported from some seventy different species of fish. Unlike *Schistocephalus*, the plerocercoid (Figs. 106, 107) is unsegmented (as in the adult strobila). It grows to a fantastic length in fish, often up to one metre. It also frequently causes parasitic castration, the cells in the pituitary being completely suppressed or greatly reduced in number (Kerr, 1948). The natural definitive hosts are fish-eating birds, and maturation takes slightly longer than *Schistocephalus*, about sixty-seven hours. In the plerocercoid the



genitalia only reach the organogeny stage of development. Maturation *in vitro* is possible, but has not been as successful as *Schistocephalus* (Smyth, 1947; 1959).

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## CHAPTER XXIII

# EUCESTODA: CYCLOPHYLLIDEA (TAENIOIDEA)

The taenioid cestodes are mainly parasites of birds and mammals, rarely occurring in reptiles and amphibians. They are characterised by possessing four well-formed hemispherical acetabula symmetrically placed around the small, rounded scolex, which is typically armed with hooks.

They differ from the pseudophyllids in that (a) the vitellarium is median, single and not follicular, (b) only one vitelline cell takes part in the formation of the egg, (c) intermediate hosts are usually terrestrial animals and rarely aquatic, (d) only the most posterior proglottids are mature, (e) they have an apolytic (p. 225) strobila.

This order contains many species of medical, veterinary or biological importance. Probably the most worked on and most easily available cestodes are the rodent species, *Hymenolepis diminuta* and *Hymenolepis nana*, both which are considered below.

### 23.1 Type Example: *Hymenolepis diminuta*

- definitive hosts: rats, mice, hamsters. Occasionally found in other mammals including man  
location: anterior ileum  
intermediate host: some thirty species of insects or their larvae. Commonly *Nosopsyllus fasciatus*, *Ctenocephaloides canis*, *Tenebrio molitor* (larva), *Tribolium confusum* (larva)  
distribution: widespread

*Morphology* (Fig. 108). The scolex bears four deep acetabula and an unarmed pyriform rostellum which can be withdrawn into a rostellar sac. The strobila measures 20–60  $\mu$  with about 1,000 proglottids, each of which is wider than long. The most striking feature, which is characteristic of the genus, is the possession of three ovoid testes, the

remaining genitalia possessing no unusual features. Gravid proglottids become free and are partly digested in the intestine where the eggs are released.

The eggs (Fig. 91) which, unlike some taenioids, retain the outer capsule (p. 91) are fairly resistant to chemicals and desiccation and remain viable in the faeces for some six months.

*Development in intermediate host.* In the laboratory the organism is best maintained using the larva of the confused flour beetle, *Tribolium confusum*, as intermediate host. Starved beetles readily ingest the eggs.

The oncosphere, presumably released by the action of digestive juices, rapidly penetrates into the haemocoel. The initial form in the haemocoel is a spherical mass

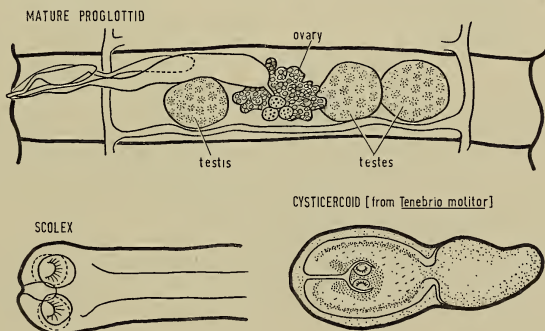


FIG. 108. *Hymenolepis diminuta*—morphology of proglottid, scolex and cysticercoid (after Chandler, 1955).

of cells in which, as it grows, appears an eccentric cavity. This cavity extends, the body elongates and becomes divided into three regions; a fore body (future scolex); a mid-body, containing the expanding cavity; a hind body, or tail. A detailed morphological description has been given by Voge and Hyneman (1957).

At 30° C., which is the optimum temperature for development in *Tenebrio molitor*, approximately 180 hours are required to reach this stage. The resulting cysticercoid (Fig. 108) is then usually infective for the rat host.

*Development in definitive host.* *H. diminuta* 'normally' develops in the laboratory albino rat, but will also develop in albino mice, hamsters and hooded rats. The size attained in each host depends on the worm load and varies with the host. In general, larger specimens are obtained from larger hosts. The average size developed in four rodent species is shown in Table 34. The pre-patent period in these hosts varies between 19–21 days.

Development of *H. diminuta* is markedly affected by the size of the infection and is roughly inversely proportional to the number of worms in the same strain of host, a result predictable on nutritional grounds.

TABLE 34  
SIZE ATTAINED BY *HYMENOLEPIS DIMINUTA* IN  
DIFFERENT HOST SPECIES  
(data from Read and Voge, 1954)

Host	Host weight (gms)	Worm vol. (ml)
Mouse . . . .	27.6	0.28
Hamster . . . .	123	0.57
Albino rat . . . .	377	1.11
Hooded rat . . . .	452	1.33

### 23.2 Family Hymenolepididae

The diagnostic features of this large family are: scolex armed with one circlet of 8-10 hooks; 1-3 large testes, uterus sacciform. Its members occur primarily in birds, but also in mammals. The genus *Hymenolepis* with three testes has over 400 species (Hughes, 1940, 1941). In addition to *H. diminuta* described above, *H. nana nana* and *H. nana fraterna* have been much investigated.

*Hymenolepis nana nana*. This remarkable 'dwarf tapeworm' of man has so far evolved in its adaptation that it can dispense with an intermediate host entirely. The size range is 25-40 mm  $\times$  1.0 mm. It is closely related to, and may be identical with, a rodent species, *H. nana fraterna*.

*Distribution*. Widespread with an incidence of 0.1-7 per cent in the southern United States and in Latin-American countries.

*Life cycle*. Development can occur with or without an intermediate host. In the direct cycle eggs passed in the faeces are ingested and hatch in the duodenum. A released oncosphere penetrates the villi of the duodenum or jejunum and rapidly undergoes development to a cysticeroid. When a cysticeroid is fully developed, it bursts out of the villi and, becoming attached firmly to the mucosa by its scolex, grows and matures to an adult strobila. The prepatent period is about fourteen days.

*H. nana* can, however, also undergo development in a number of arthropods commonly associated with rodents or rodent feeding-stuffs, for instance, *Tenebrio molitor*, *T. obscurus*, *Pulex irritans*, *Xenopsylla cheopis*, *Ctenocephaloides canis*, in which a cysticeroid similar to that of *H. diminuta* is formed. Both *H. nana* and *H. diminuta* have been used extensively for physiological studies on cestodes.

Immunity develops in the case of direct egg infections, but not after cysticercoid infections. The immunity developed as a result of egg infections is also operative for subsequent cysticercoid infections. In non-immunising cysticercoid infections it has been shown that eggs released can hatch in the intestine without being required to be re-swallowed and attacked by the battery of enzymes in the upper duodenum.

### 23.3 Family Taeniidae

This is the most important cyclophyllidean family from the point of view of containing species of medical or economic importance. With a rare exception, the worms are large and the larvae develop in homoiothermic hosts (especially carnivora and rodents).

#### 23.3I *Taeniae* of Man

Man acts as the definitive host for two species of this genus, *Taenia saginata* and *T. solium*, the so-called 'beef' and 'pork' tapeworms. Formerly, *T. solium* was commoner

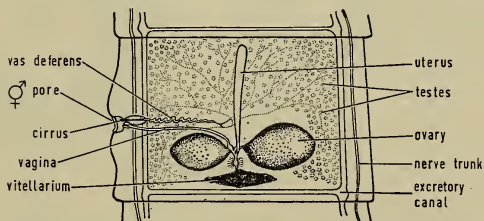


FIG. 109. *Taenia saginata*—morphology of proglottid (after Faust, 1949).

than *T. saginata*, and most elementary books of zoology dealt with it as an example of a tapeworm. In the United States and the British Isles, it has become almost extinct, and in these countries *T. saginata* is now the commonest cestode parasite of man and will be dealt with in some detail here.

#### *Taenia saginata*

**Distribution.** Has a cosmopolitan distribution in beef-eating countries, being especially prevalent in Mohammedan countries. It is relatively widespread in Great Britain and U.S.A., but precise data are not available. Silverman (1955) has reviewed bovine cysticercosis in Great Britain.

**Morphology.** An adult strobila normally reaches a length of 12–24 feet, but specimens up to 50 feet have been reported. The scolex is without a rostellum or hooks and bears only four suckers. The morphology of a proglottid is too well known to merit detailed description and is shown in Fig. 109.

The fully grown tapeworm develops 700–1,000 proglottids and sheds 3–10 of these daily; each proglottid contains about 100,000 eggs which break out when it disintegrates. Detached proglottids often show marked individual activity and may creep out through the anus on to the perianal skin. The uterus in a gravid proglottid has 15–35 lateral branches on each side, some of which bifurcate. It is usually readily distinguished from a proglottid of *T. solium* which has a uterus with only 7–10 main uterine branches (Fig. 110).

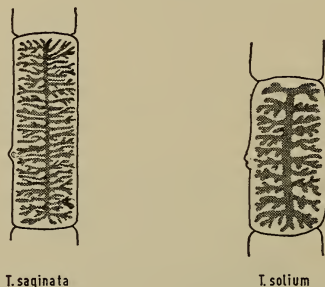


FIG. 110. Comparison of the gravid proglottids of *Taenia saginata* and *T. solium* (after several authors).

*Life cycle.* (Fig. 111). In Great Britain and the U.S.A. cattle are the normal intermediate hosts, but in the tropics several other ruminants (e.g. goat, sheep, llama, giraffe, etc.), may serve. Human infections with cysticerci are exceedingly rare.

On ingestion by a ruminant, the thick embryophore of the ova remains unaffected in its passage through the first three compartments of the stomach. On reaching the abomasum, it is exposed to the action of pepsin which attacks the cementing substance. On reaching the duodenum, it is attacked further by the pancreatic secretion, and disintegrates releasing the oncosphere, still contained within its lipoidal oncospherical membrane (Silverman, 1954a, b, c).

Pancreatic lipase, acting in the presence of bile salts and bile steroids, increase the permeability of the lipoidal membrane and activates the oncosphere. The exact factors which activate the embryo are unknown, but when activated the embryo uses its hooks to tear its way through its enclosing membrane.

Histolytic secretions released by the oncosphere assist it to bore its way through the mucosa and into the general circulation. The embryos develop especially in voluntary and cardiac muscle, but also in fat. They can, however, occur in muscles all over the body, in the diaphragm, shoulder, tongue and in other parts. Cysticerci become infec-



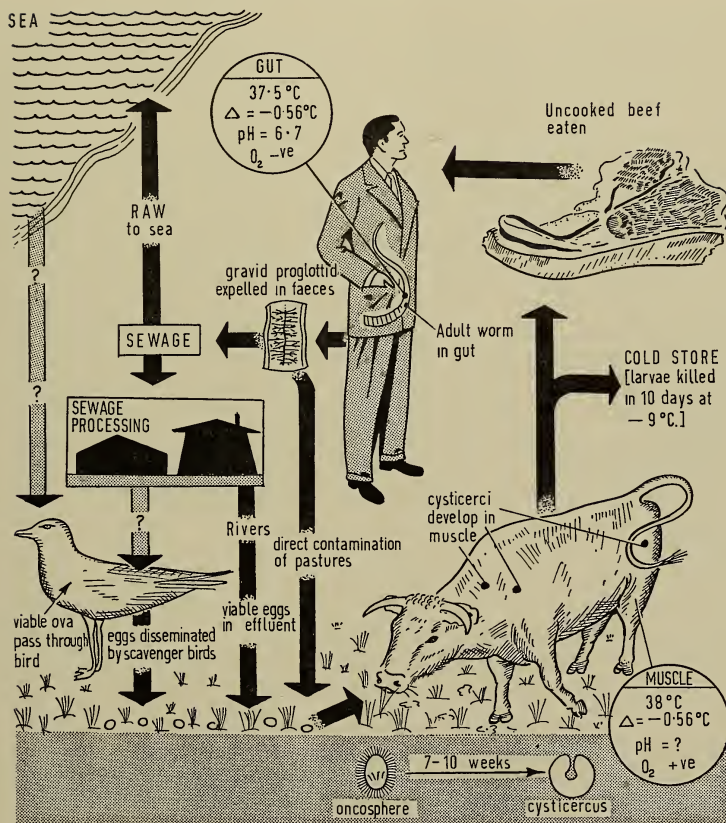


FIG. 111. Life cycle of *Taenia saginata* based on the work of Silverman and Griffiths, 1955 (original).

tive within 7-10 weeks and remain viable up to 4-9 months. After this period they are destroyed as a result of a host tissue reaction which encloses each one within a fibrous cyst.

**Infection of intermediate host.** The mode of infection of the bovine host is imperfectly

understood, as the majority of human infections occur in urban districts where the carriers have no opportunity of directly infecting pastures with egg-laden faeces. It has been shown (Silverman and Griffiths, 1955) that cestode (and other) eggs are able to negotiate the various sewage processes and may be disseminated over a wide area. Disposal of raw sewage into the sea, release of treated effluent into rivers, and use of sewage sludge as fertiliser all seem to assist in the dissemination. This is probably aided, perhaps to a considerable extent, by scavenger birds such as seagulls, for it has been clearly demonstrated that *Taenia saginata* eggs can pass through a bird gut and still retain their viability. Sea birds feeding on the shore and moving inland to roost for the night are thus potential disseminators. Eggs may remain viable and infective for as long as a year.

*Taenia solium*. This worm has a wide distribution in pork-eating countries but it is rare in Great Britain, the United States, India and the Philippines, where, indeed, specimens are difficult to obtain for laboratory study. Human infections with the cysticercus stage are commoner than with the adults, and the species may be becoming extinct.

*Morphology*. The worm is usually 9–15 feet long, that is, considerably shorter than *T. saginata*. Its scolex differs from *T. saginata* in possessing a rostellum with two rows of hooks. The morphology of a mature proglottid does not differ significantly from that of *T. saginata*, although the gravid proglottid has less lateral branches to its uterus (Fig. 110).

*Life cycle*. The life cycle is similar to that of *T. saginata* but the pig acts as intermediate host. A cysticercus, termed *Cysticercus cellulosae*, which has a white opalescent colour, may lodge anywhere in the body but shows predilection for muscles of the jaws, heart, neck, tongue, elbow, shoulders and the thighs. When 'measley pork' is eaten by man, the scolex evaginates and becomes attached to the duodenum, the 'bladder' is digested, and the strobila grows. Its rate of growth has not been studied.

*Cysticercosis*. Unlike *T. saginata*, the eggs of *T. solium* when ingested by man can develop into cysticerci and the presence of these larval stages are considerably more pathogenic to man than the adult. Cysticerci in muscles do little damage, but in sites such as the nervous system or sense organs, substantial damage may result.

*Multiceps multiceps*. A tapeworm of carnivores (especially dog, coyote, fox, jackal) with a cosmopolitan distribution. The intermediate stages develop in the brain and spinal cord of ungulates, especially sheep, and cause a disease called 'staggers' or 'gid' which affects the balancing powers of the animals. The dog is the common definitive host in Great Britain and the U.S.A.

The cysticercus possess unusual powers of asexual multiplication, forming a bladder

or *coenurus* which may give rise to many hundreds of daughter scolices directly from its inner wall (Fig. 112). It thus differs from a hydatid cyst (Fig. 92). The coenurus may similarly develop in man causing severe damage and even death, but only a dozen or so human cases have been reported.

### 23.32 Miscellaneous Taeniae

*T. pisiformis* (= *T. serrata*). This is one of the commonest tapeworms of dogs and several other wild carnivores, but it rarely occurs in the cat. In the general anatomy of its scolex and proglottids it resembles *T. solium*. Its larval stages, known as *Cysticercus pisiformis*, are well known in rabbits where they develop in the liver and mesenteries. After penetrating the intestinal wall and reaching a blood vessel, the oncospheres are carried to the liver where they grow rapidly, and after 30 days migrate to the surface of the liver, finally breaking out into the coelom. After being free in the coelom for some time, they become attached to the mesentery and become enclosed in an adventitious cyst produced by the host.

*Hydatigera taeniaeformis* (= *T. crassicollis*). Occurs in the small intestine of the cat and other carnivores, such as the stoat, lynx and fox. The scolex is characterised by possessing prominent suckers and no distinct neck region. The chief interest of this species lies in its larva, *Cysticercus fasciolaris*, which does not form a cysticercus but a *strobilocercus* (Fig. 92). This may induce sarcoma in the liver (Bullock and Curtis, 1924). The intermediate hosts are said to be rats, mice, rabbit, squirrel and muskrat, but Hutchison (1959) found that in the laboratory, development took place most readily in the mouse. On reaching the liver in the intermediate host, the strobilocercus develops and grows rapidly, becoming infective after 30 days. As Hopkins and Hutchison (1958) have shown, an unexplained relationship apparently exists between the nitrogen content and infectibility, the former dropping from 6 per cent until the infective stage is reached when it becomes stable at 4.25 per cent (Fig. 113). The prepatent period in the cat host is 16–18 days (see also p. 272).

A careful study of the growth of the adult phase of *H. taeniaeformis* has been made by Hutchison (1959). On entering the host gut, 20–70 per cent of the strobilocercus (=pseudostrobila) is shed. There is no preliminary lag period and growth commences

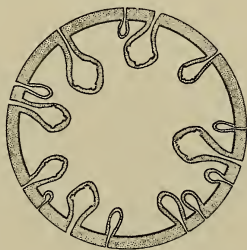


FIG. 112. Coenurus larva of *Multiceps multiceps*, showing budding of scolices from inside wall (after Benham, 1901).

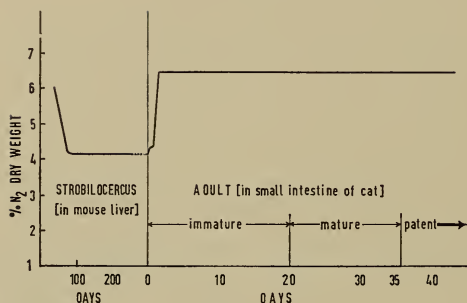


FIG. 113. Variation in nitrogen content of larval and adult *Hydatigera taeniaeformis* with age; note the gradual drop in N<sub>2</sub> composition until about the time when the strobilocercus becomes infective (63–65th day) when the level remains steady at about 4.25 per cent (adapted from Hopkins and Hutchison, 1958).

immediately on entering the small intestine. During the first 42 days development consists of two distinct exponential phases. The first has a doubling time of eight days and terminates on the 18th day after infection. There then follows a period of deceleration and growth enters a second exponential phase with a doubling time of 16 days. The transition between the two phases coincides with the onset of egg-production between 16th–18th day.

*Echinococcus granulosus*. This 'species' (see p. 264) is a small (2–8 mm long) intestinal cestode of carnivores, dogs being the commonest definitive host. Its larval stage, the hydatid, is of immense economic and medical importance and the causative agent of echinococcosis or hydatid disease. The morphology of the adult worm is shown in Fig. 114. The organisation is peculiar, for never more than three proglottids are formed, in contrast to the large numbers formed by the majority of cestodes. The rate of somatic mitosis is probably thus much lower than in these other species.

*Life cycle*. The egg is of the typical taenioid type, indistinguishable from eggs of other taenioid species.

*Hydatid*. Man and animals such as sheep, pigs, kangaroos, horses, rabbits, deer and caribou act as intermediate hosts in which the larval stage takes the form of a *hydatid* cyst (but see p. 264). The sheep is the most commonly infected. Ingested eggs make their way through the mucosa and penetrate a mesenteric



FIG. 114. *Echinococcus granulosus*. Entire strobila from dogs (after Brown, 1950).

venule or a capillary and are transported until filtered out by some favourable environmental site. The largest number of eggs become trapped in the liver, but some reach the lungs, kidneys, spleen, muscles, brain and other organs. Within about four days a miniature hydatid is formed and by 30 days it is 1 mm in diameter. In about five months, the inner surface of the cyst begins to form vesicles, the brood capsules, which frequently become separated from the mother cyst wall. Within these brood capsules, scolices develop from the wall (Fig. 92). The parent cyst sometimes develops 'daughter cysts' which then develop brood capsules. Abnormalities may occur, the brood capsules may fail to produce scolices, the hydatid may be sterilised by calcification, or for unknown reasons fail to produce brood capsules.

Cysts usually grow to 5–10 cm, but sizes up to 50 cm have been reported, and such a cyst may contain 10–15 quarts of liquid. Since each quart may contain up to 1 million scolices, the potential reproductive powers of the organisms can be imagined.

Cysts of the type whose development is described above are termed *unilocular* cysts, but two abnormal varieties are known: the alveolar and osseous hydatid.

*Alveolar* (= *multilocular*) *hydatid*. A peculiar variety of hydatid now known to be due to a different species, *E. multilocularis* (see below). It is essentially a malignant tumour with a sponge-like mass not delimited by a host capsule. There is no cystic fluid, only a jelly-like matrix.

*Osseous hydatid*. Essentially an unilocular cyst confined within a bone. Such hydatids are usually sterile but may produce scolices. The upper ends of the long bones, the vertebrae and ribs are most frequently affected.

*Pathology*. The damage caused by any type of the above cysts depends on their location and size. Enormous cysts may produce gross mechanical damage. Allergic symptoms may also develop as a result of the cystic fluid leaking. Severe leaking or bursting releases cysts to other parts of the body where they may develop into metastatic cysts.

*Biological diagnosis*. Precipitation and complement fixation tests may be used but are not altogether reliable. An intradermal reaction known as Casoni's test is more specific and dependable. It consists briefly in injecting 0.2 ml of sterile hydatid fluid intradermally, when a positive result is indicated by a wheal in 15–20 minutes; an additional delayed action at the injection site may follow.

*Distribution*. Although the life cycle of *E. granulosus* has been known for decades, hydatid disease is still widespread through the world. It is particularly prevalent in Greece, Yugoslavia, Hungary, Morocco, Australia and New Zealand, where, in endemic areas, up to 50 per cent of the sheep and cattle, and 40 per cent of the dogs, may be infected. The introduction of strict hygienic measures and their enforcement has led to the disease being virtually eliminated from Iceland, where it was once of



major importance. A useful survey of the epizootiology of *Echinococcus* is that of Gemmell (1960).

*Control.* Infections result from intimacy with dogs, or from contact with food or dishes contaminated with faeces. Dogs become infected by ingesting the entrails of sheep or cattle containing hydatids. Control methods involve careful washing after contact with dogs, the burying or burning of animal offal, and the periodic de-worming of dogs.

*Speciation.* For many years there has been controversy concerning whether or not hydatid cysts and alveolar cysts are different larval forms of the same species. Hydatid cysts are almost cosmopolitan, but in contrast, alveolar *Echinococcus* has a geographical distribution restricted to certain areas in Southern Germany, in the Alps, the Jura and in Russia and Siberia as far as the Behring Sea.

Experimental work by Vogel (1955) has shown that alveolar hydatid is almost certainly the larval form of a different species of tapeworm, now called *E. multilocularis*. The final hosts of this species are foxes, dogs and domestic cats, the natural intermediate hosts being field mice, perhaps other rodents, and occasionally man. Field mice take the eggs from fox faeces, the larval stages develop in mice, and foxes eat the mice. Man could become infected by taking up eggs from fox dung or during the skinning of foxes. Fox faeces could also infect wild fruit or vegetables.

*E. granulosus* differs from *E. multilocularis* in having herbivora as intermediate hosts. Vogel (1955) has also described the following morphological differences between the adult worms:

- |  |   |
|--|---|
| (a) In <i>E. multilocularis</i> the position of the genital pore is in front of the middle of the proglottid;                | in <i>E. granulosus</i> it is in the middle or behind this.                               |
| (b) In <i>E. multilocularis</i> the number of testes is 21-29;   | in <i>E. granulosus</i> it is 45-65 (others quote 35-53; 40-60).                          |
| (c) In <i>E. multilocularis</i> the testes lie between the posterior end of the proglottid and the region of the cirrus sac; | in <i>E. granulosus</i> they lie both in front of and behind the level of the cirrus sac. |
| (d) In <i>E. multilocularis</i> the uterus has no lateral branches;  | in <i>E. granulosus</i> it often has unbranched lateral branches.                         |
| (e) In <i>E. multilocularis</i> the length of the mature, relaxed worm is 1.4-3.4 mm;  | while <i>E. granulosus</i> is longer (5-8 mm).  |



## 23.4 Other Cyclophyllidea

*Dipylidium caninum*. A tapeworm of dogs, cats, foxes, and occasionally man, found all over the world. The characteristics of the scolex and proglottids are shown in Fig. 115. The genitalia in each proglottid are duplicated. The gravid proglottids when passed in faeces may show some activity. The dog-flea, *Ctenocephalides canis*, the cat flea, *C. felis*,

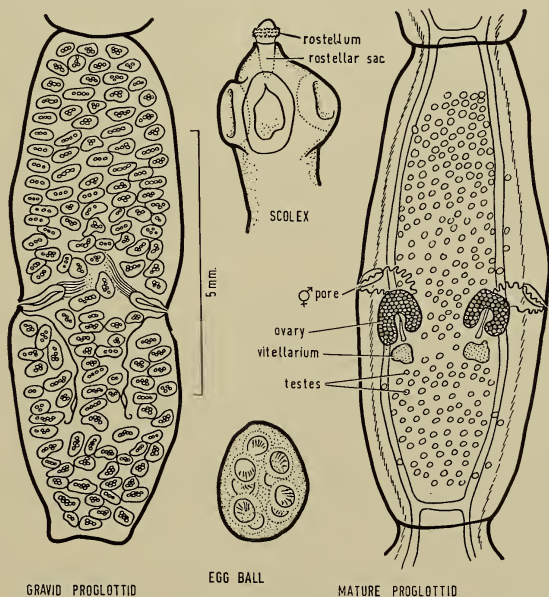


FIG. 115. *Dipylidium caninum*—morphology of scolex and proglottids (after Chandler, 1955).

the human flea, *Pulex irritans*, and the dog louse, *Trichodectes canis*, can act as intermediate hosts. Fleas become infected only in the larval stage, but a cysticercoid does not become fully developed until a flea becomes an adult. Possibly the nutritional level of the larval body cavity is insufficient to permit growth to the cysticercoid level of organisation.

Of humans, children are most commonly infected, due to ingestion of fleas or to being licked or kissed by a dog after it has nipped a flea.

Genus *Moniezia*. Two species of this genus, *M. expansa* and *M. benedeni* occur in sheep and other ruminants in many parts of the world. They are long worms (up to 600 cm) with duplicate sets of genitalia in mature proglottids. They contain well-developed interproglottidal glands of unknown function; the arrangement of these varies with the species. The life cycle of *Moniezia* remained a mystery until Stunkard in 1937 showed that cysticeroid development could occur in oribatid mites. The occurrence of the worm is seasonal in Great Britain. Lambs or sheep acquire the infection during the summer months (pre-September), and the eggs are eaten by mites on the grass. The pre-patent period in the mites is 2-5 months. The life of a worm appears to be limited to three months, an unusual characteristic of a cestode, and one probably related to the physiology of the host.

*Davainea proglottina*. A minute tapeworm of poultry (0.5-3.0 mm long), with cysticercoids in various species of slug. Widespread throughout the world.

*Raillietina tetragona*. One of the largest of fowl tapeworms measuring up to 25 cm in length. Intermediate hosts are *Musca domestica*, and ants of the genera *Tetramorium* and *Pheidole*.

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## CHAPTER XXIV

# CESTODA: PHYSIOLOGY

Before considering the physiology of cestodes in detail, attention must be drawn to certain morphological features of organisation, not encountered in other groups of helminths, but which are of major importance in understanding their metabolic processes. In discussing these features, it is considered that in the adult stage all cestodes are intestinal parasites.

(a) Cestodes lack a digestive tract and a defined circulatory system. Thus, all substances and gases must enter and leave the body through the external surface, and diffusion gradients are of undoubted significance to these organisms.

(b) Since a circulatory system and a respiratory pigment are absent, the oxygen tension will be determined by the tension in the intestine and the diffusion coefficient for cestode tissue. The oxygen tension in the central part of the strobila may thus be zero.

(c) The external surface is such that nutritional substances can be absorbed across it. The limiting dimensions of these substances are probably those of amino acids or monosaccharides.

(d) Cestodes are often of enormous length (e.g. *D. latum*) and the anterior part of the strobilia usually lies in the anterior region of the intestine which is relatively rich in nutritional materials (e.g. the duodenum), whereas the more posterior part may extend into a region relatively poor in food materials. Thus anterior and posterior regions may be absorbing at different rates.

Since the integument of cestodes must assume the entire function of absorption, it is not surprising to find that it has an unusual structure. It has been shown (Fig. 89) that in species so far examined the surface is drawn out into finger-like villi which provide a relatively enormous surface for absorption. The evidence suggests that the cestode integument has an absorptive function, possibly comparable to that of the intestinal

mucosa of higher forms, and that like it too, it performs the selective absorption of physiological substances by mechanisms that depend on an active metabolism of the organisms rather than by simple diffusion.

The physiology of this group of parasitic helminths has been more extensively studied than that of any other. This is undoubtedly due to the comparatively large size of the strobila, which makes for accuracy in quantitative measurements on a macro-scale, and to the ease with which certain species may be maintained in laboratory animals. Much recent work has centred on *Hymenolepis diminuta* (p. 254) in rats, a cyclophyllidean whose cysticeroid stage occurs in the larva of the flour beetle *Tribolium confusum*, which is easily raised in the laboratory. Some work has also been carried out on other cyclophyllids, especially those of economic or medical importance such as *Echinococcus granulosus* and *Hydatigera taeniaeformis*. Various aspects of cestode physiology have been reviewed by Smyth (1947), von Brand (1952), Bueding and Most (1953), and Read (1955).

The more readily obtainable pseudophyllids have received some attention, especially those such as *Schistocephalus solidus*, *Ligula intestinalis*, and *Diphyllobothrium spp.*, whose plerocercoids are found in fresh-water fish.

However, in no group of helminths, perhaps, have experimental results been so inconsistent or difficult to reproduce. The interactions of cestodes with their hosts are complex and not only may a species vary its metabolic behaviour in different species of host, but may do so in different strains of the same host. With a few exceptions, little unequivocal quantitative data have been obtained for this group.

### 24.1 Chemical Composition

Available information is summarised in Table 35. As emphasised elsewhere, data on the chemical analysis of parasitic worms are of little value unless qualifying information is provided on the nutritional state of the host at the time of autopsy. The carbohydrate content especially is liable to fluctuate, and as this affects the dry weight, changes in other constituents, such as protein when expressed as a percentage, may be more apparent than real. For example, Reid (1942) found that the glycogen content of *Railletina cesticillus* fell from 4.6 per cent to 0.25 per cent (wet weight) within 20 hours of host starvation, a figure reflecting the order of fluctuation in organisms parasitic in warm-blooded hosts. It is also shown (p. 271) that the glycogen content can vary with the region of the worm. With tissue of this nature then, it is clearly essential to take large samples to obtain reliable results and yet with a few exceptions (e.g. Hopkins and Hutchison, 1958) this has not been done. Some of the quantitative results discussed below may therefore be open to criticism on these grounds.

TABLE 35

## ANALYSES OF CESTODES, EXPRESSED AS A PERCENTAGE OF THE DRIED WEIGHT

The content of various constituents can vary greatly with the nutritional conditions of the host, and for these figures to be of value, the nutritional condition should be known. A number of earlier results, based on methods of doubtful validity, have been omitted; for these see von Brand (1952)

Species	Dry matter as % fresh weight	Chemical analysis (% dry wt.)			Stage	Reference
		Carbo-hydrate (glycogen)	Lipid	Protein <sup>1</sup>	Inorganic substances	
<i>Diphyllobothrium dendriticum</i>	29.9	31.5	—	41	—	Archer and Hopkins (1958)
<i>Diphyllobothrium dendriticum</i>	30.8	36.2	—	47	—	Archer and Hopkins (1958)
<i>Schistocephalus solidus</i>	31.8	50.9	—	35.8	5.8	Hopkins (1950)
<i>Schistocephalus solidus</i>	38	28.0	—	—	—	Hopkins (1960)†
<i>Ligula intestinalis</i>	29.0	38-52	—	35-45	—	Markov (1939)*
<i>Raillietina cesticillus</i>	20.5	30.2	15.5	33.1	11.5	Reid (1942)*
<i>Moniezia expansa</i>	10-11	24-52	30.1	30-36	9.3-10.5	von Brand (1933)*; Wardle (1937)*; Weinland (1901a)*
<i>Multiceps multiceps</i> ( <i>Coenurus cerebralis</i> )	25.3	—	—	—	27.4	Schopfer (1932)*
<i>Multiceps multiceps</i> ( <i>Coenurus cerebralis</i> )	12.4	—	—	—	4.1	Schopfer (1932)*
<i>Taenia hydatigera</i>	23.5	28	4.9	—	—	von Brand (1933)*
<i>Anoplocephala magna</i> ( <i>Taenia plitata</i> )	27.5	6	33.1	—	—	von Brand (1933)*
<i>Hydatigera taeniaeformis</i>	—	28.0	5.8	32.2	16.3	Salisbury and Anderson (1939)
<i>Hydatigera taeniaeformis</i>	28	44	5.3	27	18.1	Hopkins (1959)
<i>Hydatigera taeniaeformis</i>	—	29-41	—	40.6	—	Hopkins and Hutchison (1958); Hopkins (1959)
<i>Hymenolepis diminuta</i>	17	32-63	—	—	—	Read (1956)
<i>Echinococcus granulosus</i>	14.8	18.8	13.5	62.1	13.5	Agoston <i>et al.</i> (1957)

\* References given in Smyth (1947)

† Personal communication

<sup>1</sup> Protein calculated as from total N  $\times 6.25$



*Carbohydrates.* As with trematodes (p. 212) and nematodes (p. 249), the main polysaccharide in cestodes is glycogen. It is known that in vertebrate muscle, glycogen exists in two states, trichloroacetic acid soluble and trichloroacetic acid insoluble, the former being that most readily available to meet the metabolic demands of the body and the first to disappear during exercise. This question has not been investigated in many cestodes, but it is known that in *Hymenolepis diminuta* the trichloroacetic acid soluble fraction represents approximately 80 per cent of the total glycogen of the worm (Daugherty and Taylor, 1956). Data are not available for other species.

The data presented in Table 35 show that the total glycogen content of cestodes may vary within the range of 6–63 per cent of the dried weight. In general, larval cestodes show a higher and relatively more constant glycogen content than the corresponding adults. This reflects the more stable intermediate host environment, usually the coelomic cavity or tissues.

In different strains of the same host, the *initial* glycogen reserves of specimens removed for experimental work may show enormous variation, although the actual consumption of glycogen *in vitro* (under aerobic conditions) is similar. Thus Read (1956) reported the glycogen content of *Hymenolepis diminuta* was 32 per cent of the dried weight, in the strain of rats used at one Institution (the Rice Institute), whereas from specimens used at another Institution (University of California) a figure of 63 per cent was obtained. These figures reflect the difficulties of attempting to obtain accurate quantitative data on cestode metabolism.

The glycogen content may also show further fluctuation depending on the region from which the sample has been taken. In *H. diminuta*, for example, the glycogen content of different quarters of a strobila varies from 21–44 per cent of the dried weight (Table 36).

It is clear, then, that the glycogen content of a strobila, or a particular part of it, may depend on a number of factors, the majority of which are very imperfectly understood. Any quoted figures for the carbohydrate content should thus be accepted with caution, unless the previous metabolic history is known, and the region of the strobila defined.

*Protein.* Cestodes are unique in the animal kingdom in that, with a few exceptions, their protein content is less than the sum of the glycogen and fat. Analysis shows the protein content lies in the range 27–62 per cent of the dry weight (Table 35).

Analyses for protein are usually based on the total nitrogen content, the protein being calculated by multiplying by 6.25. There is some evidence that the non-protein nitrogen fraction in cestodes may be high and if this is generally confirmed, the usual conversion factor of 6.25 will require revision for cestodes.

Most of the analyses are based on a relatively small number of specimens, and possible variables such as size and age of worms have not been taken into account. That these can be important has been shown by a careful study by Hopkins and Hutchison (1958) on the nitrogen fraction of *Hydatigera (Taenia) taeniaeformis*. Basing their conclusions on a large amount of material, they have shown that strobilocercus larvae of the same age have the same percentage  $N_2$  composition except in the case of heavy infections of over 100 worms. During growth of the strobilocercus the nitrogen content falls to a constant level ( $4.25 \pm 0.25$  per cent dried weight) 67 days after the initial infection of the mouse. This period corresponds almost exactly with the earliest time (63 days) at which

TABLE 36

THE GLYCOGEN CONTENT OF LINEAR QUARTERS OF THE STROBILA  
OF *H. DIMINUTA*

Values expressed as per cent of the dried weight (recalculated from data of Read, 1956)

Worm No.	Anterior quarter	Second quarter	Third quarter	Posterior quarter
1	21.0	44.2	42.9	40.2
2	23.6	42.7	43.0	26.4
3	22.5	44.8	40.5	27.0

larvae have been found to be infective to the definitive cat host; it is reasonable to assume that infectivity and nitrogen level are linked although the nature of the relationship is, as yet, unknown. When the worm becomes established in the cat's intestine, a sharp rise in the  $N_2$  level to  $6.4 \pm 0.7$  per cent dried weight occurs and this level is maintained throughout the 36 day prepatent period. Results such as these illustrate the importance, repeatedly stressed throughout this text, of correlating analytical figures with a particular stage and age in the life of a parasite.

Fractionations of proteins have been carried out on several species such as *Moniezia expansa*, *Taenia saginata*, *Hymenolepis diminuta*, *Raillietina cesticillus*. In these species probably less than 1 per cent of the protein fractions are pure protein (Kent, 1947, 1957a, and 1957b); the remainder occurs as conjugated proteins linked with other tissue substances such as glycogen, cerebrosides, or bile acids.

Analysis of *Hymenolepis diminuta* may be taken as typical of the results obtained.

In *H. diminuta* four main protein complexes have been isolated and designated  $P_1\alpha$ ,  $P_1\beta$ ,  $P_2\alpha$ ,  $P_3$ ; a very small fraction  $P_4$  was also isolated.  $P_1\alpha$  and  $P_1\beta$  are water soluble at pH 7.  $P_1\alpha$  contains 92.2 per cent protein and 6 per cent carbohydrate;  $P_1\beta$  contains 78.7 per cent protein, 15 per cent carbohydrate and 6 per cent cerebrosides. Both these fractions are electrophoretically homogeneous at pH 8.6. The  $P_2\alpha$  fraction contains 61.5 per cent protein, 0.41 per cent phosphorus and 28.4 per cent carbohydrate.  $P_3$  is

a nucleo-protein complex extracted with 10 per cent NaCl and contains 75 per cent protein, 1 per cent phosphorus and 10 per cent carbohydrate. Composition of the 5th fraction has not been studied.

The significance of these protein complexes or their fractions in the tissues of the worms has not been determined, but the results of analysis suggest an interrelation between the carbohydrate and protein metabolisms closer than that normally occurring in other organisms.

The proteins of *Moniezia expansa* and *Hymenolepis diminuta* have been subjected to amino acid analysis, but this has revealed nothing unusual. In *Hymenolepis diminuta*, 20 amino acids have been identified but never more than 16 have been found in any one hydrolysate, and it has been shown that there exists a close parallel between the amino acid and concentrational distribution in the worm and the rat host intestinal tissue (Goodchild and Wells, 1957). This may indicate that these parasites are capable of extracting needed protein precursors from the mucosa of the intestine while in intimate contact with it.

**Lipid.** The lipid content of cestodes shows great variation, ranging from 4.9 per cent (dry weight) in *Taenia hydatigera* to 30 per cent (dry weight) in *Moniezia expansa*. Only a few detailed fractionations have been made and these have shown that all the usual fractions occur, phospholipids, cerebroside, unsaponifiable material, fatty acids and glycerol. The fatty acids are chiefly by-products of the carbohydrate metabolism, and will be dealt with under that heading (p. 277).

Detailed fractionations have only been carried out in the case of the larvae of *Hydatigera taeniaeformis*, *Diphyllobothrium latum* and *Moniezia expansa*.

(a) **Phospholipids.** In a phospholipid, the glycerol is attached to two fatty acid molecules and a molecule of phosphoric acid; the latter is also combined by another ester linkage with an organic base. Phospholipids thus behave as zwitterions, since they contain both acid and basic groups. Phospholipids possess both water attracting (hydrophilic) properties and fat attracting (hydrophobic) properties, and are soluble in both water and fats; they therefore serve the useful role in cellular metabolism of binding these two types of compounds together. In larval *Hydatigera taeniaeformis* (*Cysticercus fasciolaris*), phospholipids represent about 30 per cent of the total lipids, and consist of a mixture of lecithin and cephalin. Cleavage products of the hydrolysis gave about equal parts saturated and unsaturated acids. The solid saturated acids were mainly palmitic, stearic and arachidic; the unsaturated acids, after catalytic reduction, yielded mainly stearic but also a small amount of palmitic acid. In *Diphyllobothrium latum*, cephalin and lecithin fractions have also been found.

(b) **Cerebrosides.** Cerebrosides have been found in *Cysticercus fasciolaris*, *Moniezia expansa* and *Diphyllobothrium latum*.

(c) **Unsaponifiable material.** In *Cysticercus fasciolaris* and *Moniezia expansa*, unsaponifiable material giving sterol reactions has been found. This may be cholesterol but there is insufficient evidence on this point.

**Morphological distribution of fat.** Only routine staining methods have been used to determine the distribution of lipids in cestodes and no intensive study using modern

histochemical techniques has been made. In general, the parenchyma is the main storage site, but traces occur in most organs. In several organisms, fat droplets have been found in the excretory canals, and there seems little question that they represent metabolic waste products resulting from a predominantly anaerobic metabolism.

This may be demonstrated histochemically in members of the family Diphyllbothriidae. In fresh plerocercoids of *Schistocephalus solidus* or *Diphyllbothrium* sp., the parenchymal spaces are filled with glycogen and are almost fat-free. During *in vitro* culture, under starvation conditions (i.e. non-nutrient salines), the glycogen disappears from the parenchyma and cytoplasmic lipid appears. It is tempting to conclude that the fat has been formed in the sites of glycogen degradation as a metabolic waste product. This hypothesis is not unreasonable, for cestode proglottids have a short life and fatty deposits of this nature would be unlikely to be harmful during the brief period of maturation and egg-production.

## 24.2 Nutrition

As in the case of many other helminths, the nutritional demands of cestodes may change considerably during the life cycle, being correlated with a change of habitat and environment. For example, the demands of the cysticeroid larva of *Hymenolepis diminuta* undergoing only a tissue phase of development in the haemocoel of larval *Tenebrio molitor* at 25° C. will be considerably less than that of an egg-producing adult of the same species in the rat intestine at 38° C.

Only the carbohydrate requirements have been studied in detail, and these will be discussed later under carbohydrate metabolism. Briefly cestodes can metabolize only a very limited number of monosaccharide sugars (chiefly glucose and galactose), and, with the exception of *Cittotaenia* sp. which has been reported to metabolise sucrose, are incapable of utilising disaccharides.

The difficulties of obtaining unequivocal results from experiments on cestodes *in vivo* may be strikingly demonstrated from experiments on the effect of feeding the host with diets containing different carbohydrates on the growth of *Hymenolepis diminuta* (Read and Rothman, 1957). In rats containing glucose as a source of carbohydrate, growth was considerably less than in worms grown in rats containing starch as the source of carbohydrate. This result has been interpreted as being due to the fact that free glucose is more rapidly absorbed by the host (and therefore less available to the cestode), than glucose made available from enzymatic degradation of starch. Such results emphasize the need for metabolic studies *in vitro* under axenic conditions.

Lack of adequate carbohydrate in the diet results in stunting of growth, a retardation of egg-production or the production of morphologically abnormal eggs in the case of *H. diminuta*.

Very little is known concerning the protein requirements of cestodes. In this respect, *in vivo* experiments have again been unsatisfactory in providing information. Complete elimination of protein from the diet of the rat host has apparently no measur-

able effect on the establishment, growth or reproductive capacity of *Hymenolepis diminuta* (Chandler, 1943). This rather unexpected result suggests that in some way the parasite is able to satisfy its quite considerable protein requirements directly from the host tissue. It is probable that the strobila, being in close apposition to the intestinal wall, is capable of absorbing nitrogenous exudates from the mucosal cells. Alternatively, a cestode strobila may secrete proteolytic enzymes capable of breaking down the mucoid intestinal secretion into peptones or amino acids of absorbable dimensions, but such a phenomenon has never been demonstrated.

There is no evidence that cestodes have any fat requirements at all, although this aspect of their physiology has not been much investigated.

There is some evidence (Addis, 1946; Beck, 1952) that in some species sex hormones may be essential for growth and normal maturation. Thus the rate of growth and the egg-production of *H. diminuta* declines in castrated male rats, but the administration of testosterone and progesterone restores the normal conditions.

Following the general pattern of the nutrition of other metazoans, it is fairly certain that a number of accessory growth factors will be shown to be necessary for the normal metabolic processes of cestodes. It has been claimed that *H. diminuta* is independent of vitamin B<sub>1</sub> in the diet of the host for normal establishment and growth, independent of vitamins A, D<sub>1</sub> and E for growth, but dependent on the fat soluble vitamins for normal establishment. The worm is also dependent on some factor or factors present in brewers yeast for normal establishment and growth.

It is well known that the presence of *Diphylobothrium latum* in man and experimental animals is often associated with anaemia in the host. It has been shown (Nyberg, 1958) that *D. latum*, as compared with other organisms, contains large amounts of vitamin B-12. It was found that this cestode absorbs an average of about 44 per cent of a single oral dose of radioactive B-12 (Co<sup>60</sup>) the radioactivity being concentrated mainly in the proximal part of the worm. This vitamin must thus be considered a likely growth requirement of *D. latum*. In contrast, the strobila of *T. saginata* does not take up this vitamin from the host.

### 24.3 Respiration

As emphasised elsewhere (p. 268), the lack of an alimentary tract results in the exchange of gases taking place through the strobila surface. Although the lumen of the intestine is considered to be essentially anaerobic the application of the microelectrode technique by Rogers (1949) has shown that the oxygen tension in local areas of the intestinal mucosa of a rat may be as high as 30 mm of Hg. This figure is only 10 per cent



below that of the venous blood of man and is higher than that of mammalian skeletal muscle (20 mm). It is possible therefore that the external surface of a tapeworm strobilus may be in a region of sufficiently high tension to permit aerobic metabolism.

The most detailed studies on respiration have been carried out on *Hymenolepis diminuta* (Read, 1956) and *Echinococcus granulosus* (Agosin *et al.*, 1957), but a number of earlier observations have been carried out on *Moniezia*, *Diphyllobothrium* and *Trienocephorus*. Although the respiratory pattern is not the same in all the species studied, it is possible, nevertheless, to make some generalised statements.

Cestodes appear to have a predominantly anaerobic metabolism, but experimental worms consume oxygen if it is available and there is an increased consumption following anaerobiosis. Cestodes thus build up an oxygen debt, which implies that some substrates formed anaerobically are retained in the tissues and are available for oxidation during subsequent aerobic periods. In media lacking glucose, the R.Q. is between 0.5–0.9, but the R.Q. generally shows a marked increase in the presence of glucose. The aerobic respiration is dependent on the oxygen tension in the lower ranges (2–5 vol. per cent) but less so at higher tensions. Under anaerobic conditions, CO<sub>2</sub> is produced in some cases (*E. granulosus*) but not in others (*H. diminuta* and *Oochoristica symmetrica*).

Oxygen consumption of adult cestodes is inhibited to a varying extent by cyanide but never completely. The scolices of *Echinococcus granulosus*, for example, show a 47 per cent inhibition. This clearly points to the presence of heavy metal catalysts and the absorption bands of cytochrome C have been detected in a number of species. Such results must be accepted with caution as they do not necessarily prove the existence of the cytochrome system in such forms. They merely indicate that the tissues investigated contain haematin-like pigments, but whether these pigments have the same enzymatic properties as the cytochromes has not been determined.

The fermentative nature of the respiratory system in cestodes is further indicated by the fact that the major excretory product (p. 278) is lactic acid, and that the production of this acid is about 30 per cent greater in some instances (e.g. *H. diminuta*; Read 1956) under anaerobic than aerobic conditions.

There have been few studies on the respiration of larval cestodes. The aerobic respiration of the eggs and plerocercoids of *D. latum* are totally inhibited by cyanide.

It has been suggested (Read, 1956) that although the surface of a strobila may be in contact with oxygen from the mucosa, the oxygen tension in the central tissues of a large part of the strobila must be essentially zero. The end products of the fermentative type of metabolism in the central region would diffuse outwards and be available for further oxidation in the peripheral tissues. Such a mechanism might have advantages



in terms of useful energy, particularly since certain systems with high energy requirements (the muscular and nervous systems) are located in these peripheral regions.

#### 24.4 Carbohydrate Metabolism

From a series of studies carried out on both cyclophyllidean and pseudophyllidean cestodes it is possible to build up a general picture of the carbohydrate metabolism. These experiments fall into two groups (a) *in vivo* experiments: carried out by feeding the host on experimental diets and observing the effect on the chemical composition of a worm; (b) *in vitro* experiments: concerned in investigating the effect of aerobic and anaerobic conditions on the chemical composition and respiratory exchanges during short periods of incubation in saline, by means of the conventional Warburg technique. Only a few observers (e.g. Hopkins, 1952) have carried out such experiments under sterile conditions *in vitro*.

It has been shown (Table 35) that cestodes may possess relatively high glycogen reserves (up to 52 per cent in *Ligula plerocercoids*) suggesting the importance of the carbohydrate metabolism to the worm. Under experimental starvation of the host, glycogen reserves drop rapidly, as already shown for *Raillietina cestillus* (p. 269), and, even under normal feeding conditions of a host, marked daily fluctuation of carbohydrate content may take place. The release of proglottids during certain times of the day only, may be related to diurnal variations in metabolism.

In the case of *Hymenolepis diminuta*, the quality and quantity of the carbohydrate has a number of effects on the growth, size and egg-production of the worms. Read *et al.* (1950-58) particularly have studied the problem and have shown that growth and egg-production of worms is greater in rats fed on starch than in rats fed on disaccharides or monosaccharides. As explained earlier (p. 274) this result is probably due to the fact that both monosaccharides and disaccharides are more readily absorbed by the host than starch.

Although no other cestode has been studied in such detail as *H. diminuta* the general pattern appears to be the same in other species; there is a marked dependence on adequate carbohydrates in the diet.

*In vitro* studies have provided a more precise indication of the carbohydrate metabolism (Read, 1956; Laurie, 1957; Agosin *et al.*, 1957). These show that glucose and galactose (but not fructose, lactose, maltose or trehalose) are actively absorbed and any in excess of immediate metabolic requirements is stored in the parenchyma as glycogen. In many species, and particularly in larvae with cold-blooded hosts, high glycogen reserves are built up. The glucose consumption for the few species studied is

given in Table 37. In the absence of glucose in the culture medium, use is made of endogenous reserves. Under anaerobic conditions neither *H. diminuta* nor *Oochoristica symmetrica* produce any metabolic gas. However, the scolices of *Echinococcus granulosus* have been found to produce  $\text{CO}_2$  but its origin has not been elucidated (Agosin *et al.*, 1957).

In the case of *H. diminuta* at any rate, the carbohydrate consumption appears to be independent of the quantity of exogenous glucose between the limits of 0.005M and 0.066M (Read, 1956). The rate at which glucose is removed from the medium is also

TABLE 37

CARBOHYDRATE CONSUMPTION OF CYCLOPHYLLIDEAN CESTODES  
(in gm per 100 gm wet weight at 37–41° C. under anaerobic conditions)

<i>Raillietina cesticillus</i>	. 4.8	Reid (1942)
<i>Moniezia expansa</i>	. 1.0	von Brand (1933)
<i>Hymenolepsis diminuta</i>	. 5.0	Read (1956)

independent of the  $\text{O}_2$  tension over the range (0–21 per cent  $\text{O}_2$ ). A 'Pasteur effect' occurs, and in terms of carbohydrate catabolised more is utilised under anaerobic than aerobic conditions, as witnessed by the greater glycogen build-up under aerobic conditions.

All the evidence suggests that cestodes possess a phosphorylative glycolytic system resembling in part at least that which occurs in vertebrate tissues and yeast, although the metabolic pathways have only been very incompletely worked out. Detailed analysis of *H. diminuta* (Read, 1951) has shown that the tissues of this cestode contain significant amounts of materials which either closely resemble, or are identical with those compounds known to be intermediates in the vertebrate Embden-Meyerhof Cycle (Table 38). Confirmatory evidence that phosphorylative glycolysis is proceeding is given by the detection of phosphorylase, phosphohexomutase, hexokinase, aldolase, phosphoglyceraldehyde dehydrogenase and lactic dehydrogenase, a total of six out of the thirteen enzymes known to be involved in the cycle. The respiratory exchanges of cestodes are also markedly inhibited by inhibitors of glycolysis.

In all cestodes studied, lactic acid has been found to form a major part of the acidic end-products although fatty acids have also been reported. In *H. diminuta* 37–98 per cent of the excretory products are lactic acid (Laurie, 1957) and in *Echinococcus granulosus* it accounts for 50 per cent of the utilised carbohydrates (Agosin *et al.*, 1957).

There is no evidence that a Krebs's tricarboxylic acid cycle occurs in cestodes, although only limited work has been carried out in this field.

TABLE 38

## EMBDEN-MYERHOF PATHWAY OF ANAEROBIC GLYCOLYSIS IN MUSCLE

## Reaction

1.  $\text{glycogen} + n\text{H}_3\text{PO}_4 \xrightleftharpoons[\text{Mg}^{++}]{\text{phosphorylase}^1} n\text{glucose-1-phosphate}$
  2.  $\text{glucose-1-phosphate} \xrightleftharpoons{\text{phosphoglucomutase}} \text{glucose-6-phosphate}$
  3.  $\text{glucose-6-phosphate} \xrightleftharpoons{\text{phosphohexomutase}} \text{fructose-6-phosphate}$
  4.  $\text{fructose-6-phosphate} + \text{ATP} \xrightleftharpoons[\text{Mg}^{++}]{\text{phosphofructokinase}} \text{fructose-1, 6-diphosphate} + \text{ADP}$
  5.  $\text{fructose-1, 6-diphosphate} \xrightleftharpoons{\text{aldolase}} \text{dihydroxyacetone phosphate} + \text{glyceraldehyde-3-phosphate}$
  6.  $\text{glyceraldehyde-3-phosphate} \xrightleftharpoons[\text{isomerase}]{\text{phosphotriose}} \text{dehydroxyacetone phosphate}$
  7.  $\text{glyceraldehyde-3-phosphate} + \text{H}_3\text{PO}_4 + \text{DPN}^+ \xrightleftharpoons[\text{1, 3-diphosphoglyceric acid} + \text{DPNH} + \text{H}^+]{\text{glyceraldehyde-phosphate dehydrogenase}^2}$
  8.  $\text{1, 3-diphosphoglyceric acid} + \text{ADP} \xrightleftharpoons{\text{transphosphorylase}} \text{3-phosphoglyceric acid} + \text{ATP}$
  9.  $\text{3-phosphoglyceric acid} \xrightleftharpoons[\text{acid mutase}]{\text{phosphoglyceric}} \text{2-phosphoglyceric acid}$
  10.  $\text{2-phosphoglyceric acid} \xrightleftharpoons[\text{Mg}^{++}]{\text{enolase}^3} \text{2-phosphoenolpyruvic acid} + \text{H}_2\text{O}$
  11.  $\text{2-phosphoenolpyruvic acid} + \text{ADP} \xrightleftharpoons[\text{K}^+]{\text{transphosphorylase}} \text{enolpyruvic acid} + \text{ATP}$
  12.  $\text{enolpyruvic acid} \xrightleftharpoons[\text{[spontaneously?]}]{\text{ketopyruvic acid}}$
  13.  $\text{pyruvic acid} + \text{DPNH} + \text{H}^+ \xrightleftharpoons[\text{dehydrogenase}]{\text{lactic}} \text{l-lactic acid} + \text{DPN}^+$
- Overall reaction:  $[\text{C}_6\text{H}_{10}\text{O}_5] + 3\text{ADP} + 3 \text{ phosphate} \longrightarrow 2 \text{ lactic acid} + 3\text{ATP} + 2\text{H}_2\text{O}$

<sup>1</sup> inhibited by phlorizin.<sup>2</sup> inhibited by iodoacetate.<sup>3</sup> inhibited by fluoride.

### 24.5 Protein Metabolism

It is evident from the remarkable growth rate of some species that rapid protein synthesis takes place, but it must be borne in mind that cestodes could be considered as being made up, at least in part, of a string of embryos at different stages of development. When this point is considered their rate is similar to that of other embryos at the same temperature. *Hymenolepis diminuta*, for example, grows from a cysticeroid to a

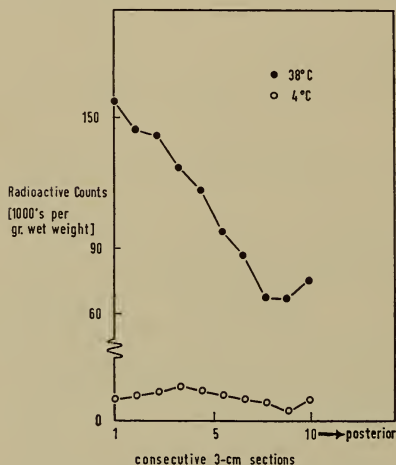


FIG. 116. Regional absorption of  $S^{35}$  methionine; note that at  $38^{\circ}\text{C}$ . the anterior end showed the higher rate of absorption (after Daugherty and Foster, 1958).

strobila some 35 cm long in fourteen days. The strobilocercus of *Hydatigera taeniaeformis* increases its body weight by approximately 120,000 times in the first forty days of its development in the mouse (Hopkins and Hutchinson, 1958). *D. dendriticum* also grows at a fast rate (Fig. 100). However, information on the intermediate protein metabolism is exceedingly meagre, and attempts to detect some of the enzymes concerned have not been satisfactory with present methods.

Complete elimination of protein from the diet of the rat host has no effect on the establishment, growth or reproductive capacity of *Hymenolepis diminuta* (p. 274). It has also been shown that the amino acid content of *Hymenolepis diminuta* and its host tissue are, on the whole, similar (p. 273).

Studies with labelled  $S^{35}$  L-methionine and  $S^{35}$  L-cystine have shown that both *Hymenolepis diminuta* and *Raillietina cesticillus* actively absorb these amino acids; absorption occurs at a relatively higher rate at the anterior than the posterior end (Fig. 116). *R. cesticillus* absorbs amino acids 4–6 times quicker than *H. diminuta*. Absorption is considerably more rapid at the temperature of the host than at lower temperatures (Daugherty and Foster, 1958). Proteolytic enzymes have been reported from *Dipyllobothrium latum*, *Taenia solium*, *T. saginata*, *Dipylidium caninum*, and *Moniezia expansa*, but evidence regarding the degree of activity of these enzymes is conflicting.

The end products of protein metabolism have been very imperfectly studied. Ammonia, urea, uric acid, creatinine and betaine have all been detected in various cestodes. The occurrence of ammonia is not surprising, for it occurs not only in *Ascaris* and *Fasciola*, but in free-living invertebrates, such as polychaetes, sipunculoides, leeches, crustaceans, echinoderms and cephalopods, all animals which can rapidly dispose of it.

## 24.6 Lipid Metabolism

This has been very little studied in cestodes. Lipases with low activity have been found in *Hydatigera taeniaformis* and *Taenia pisiformis*. Large quantities of fat frequently appear in cestodes cultured *in vitro* and may represent a by-product of the carbohydrate metabolism (p. 274). The fat soluble vitamins may be necessary for establishment in the host intestine (p. 275), but with this exception there is no evidence to indicate that cestodes have any fat requirements.

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## CHAPTER XXV

### NEMATODA:

### GENERAL ACCOUNT

The nematodes, or 'round worms', make up a large assemblage of worms of relatively simple structure with a widespread distribution, their cylindrical non-segmented bodies distinguishing them easily from other helminths. They occur in fresh water, in the sea, in soil, and are amongst the most successful parasites of plants and animals. Most of the free-living nematodes are microscopic, as are many of the parasitic species invading the body fluids such as the blood or lymph channels of their hosts. Those species which live in the intestine are generally larger, while some in tissue habitats (e.g. the kidney) grow to relatively enormous lengths.

Nematodes exhibit a wide range of feeding habits. Many feed entirely on the micro-organisms present in decaying vegetable matter, others live on the outsides of plants and suck their juices, while others venture within the plant and wander destructively through their tissues. In vertebrates, they may parasitise the eye, mouth, tongue, alimentary canal, liver, lungs or body cavity, often causing destructive and revolting diseases and producing untold hardship. A much-quoted figure, given by Stoll (1947) in an entertaining survey of helminth parasites, estimates that in a world of 2,200 million inhabitants there are some 2,000 million human nematode infections, a tribute, indeed, to the efficiency of nematode life cycles.

The life cycles range from the very simple to the extremely complicated. Most nematodes are dioecious, producing eggs with tough resistant coverings. Some species are monoecious while in some dioecious species the males are rare or as yet unknown. The monoecious species may be either parthenogenetic or self-fertilising hermaphrodites. The majority of nematodes are oviparous, but some are ovoviviparous (see p. 298). The successful development of nematode eggs outside the hosts is largely dependent on environmental conditions, particularly oxygen and temperature. All hatched

nematode larvae, whether they hatch in water, soil or within an animal host, must undergo a series of four ecdyses before reaching maturity.

### 25.1 Classification

An agreed classification of nematodes has yet to be devised and the whole field of nematode taxonomy is in an uncertain position. This situation has arisen largely on account of the separate study of free-living and parasitic groups. Recent classifications have attempted to include both groups. The classification below is based mainly on Chandler (1955) and Chitwood and Chitwood (1950). Nematodes may be divided broadly into two groups, the *Aphasmidia* and the *Phasmidia*, depending on whether caudal sense organs, the *phasmids* (p. 291), are present or not. Unfortunately, phasmids are often difficult to detect in living or preserved specimens, so that from a practical point of view, the division is rather unsatisfactory. Only those groups of interest to a parasitologist are included below.

#### Subclass I. *Aphasmidia* (= *Adenophorea*)

Phasmids absent, amphids circular, spiral, shepherd's crook, pocket-like or pore-like. Excretory system absent or reduced to a single ventral cell in cervical region. Caudal glands typically present. Mainly aquatic forms, with some terrestrial and some parasitic in vertebrates and invertebrates.

Sub-order 1. *Trichurata* (Capillarids). \* Pharynx a long fine tube embedded for most of its length in a column of glandular cells. Females with only one ovary; males with one spicule or none (e.g. *Trichuris*, *Trichinella*, *Trichosomoides*).

Sub-order 2. *Diectophymata*. Large worms parasitic in the kidneys of vertebrates. Female with one ovary; male with cup-shaped bursa-like expansion with rays (e.g. *Diectophyma*).

#### Subclass II. *Phasmidia* (= *Secernentea*)

Phasmids present. Amphids simple pores. Excretory system well developed with terminal excretory duct cuticularised. Caudal alae or cuticular bursae commonly present. Male with paired genital papillae. Six (or less) coelomocytes. Caudal glands absent. Saprophages in soil (rarely water) and parasitic in vertebrates and invertebrates.

Sub-order 3. *Rhabditata* (Rhabditids). \* Small transparent meromyarian worms. Pharynx usually with a posterior bulb and frequently a prebulbar swelling. Mouth with three to six lips. Females oviparous or viviparous and may be parthenogenetic or

hermaphroditic. Majority free-living, some with both free-living and parasitic phases (e.g. *Rhabditis*, *Strongyloides*).

Sub-order 4. *Ascaridata*. Mouth with three lips (one dorsal and two sub-ventral); pharynx bulbed or cylindrical; vagina elongate; male usually with ventrally curled tail and two spicules; alae may be present.

*Superfamily* 1. *Ascaridoidea* (Ascarids).\* Ventro-lateral cephalic papillae well developed; pharynx with or without bulb. Mainly large, stout, polymyarian worms (e.g. *Ascaris*, *Ascaridia*).

*Superfamily* 2. *Oxyuroidea* (Oxyurids).\* Ventro-lateral cephalic papillae absent; pharynx terminated by valved bulb; small or medium-sized transparent meromyarian worms; tail of female usually pointed (e.g. *Enterobius*, *Aspicularis*).

Sub-order 5. *Strongylata* (Strongyles).\* Male with copulatory bursa; mouth simple without lips, frequently with a buccal capsule and teeth; usually meromyarian; pharynx muscular, club-shaped or cylindrical (e.g. *Ancylostoma*, *Nippostrongylus*, *Syngamus*.)

Sub-order 6. *Spirurata*. Pharynx composed of two parts, both cylindroid, an anterior muscular portion and a posterior glandular portion; males usually with two spicules and well developed alae on spirally coiled tail; mouth with no lips, rudimentary lips, or with two or four paired lips. Long, thin nematodes, parasitic in vertebrates in adult stage. Mainly viviparous.

*Superfamily* 1. *Spiruroidea* (Spirurids).\* Mouth usually with a sclerotinised vestibule and two or four paired lips; males with spirally coiled tail and with broad alae supported by papillae and with spicules usually unequal and different from each other; eggs contain larvae when laid (e.g. *Gongylonema*, *Habronema*).

*Superfamily* 2. *Filaroidea* (Filaria).\* Slender, delicate worms; mouth without lips and rarely with a vestibule; vulva near anterior end of body; usually viviparous. Tissue parasites of vertebrates, except fish (e.g. *Wuchereria*, *Onchocerca*).

Sub-order 7. *Camallanata*. Mouth simple or with two lateral 'jaws'; posterior part of pharynx with one to three large nuclei; intermediate host, copepods.

*Superfamily* 2. *Dracunculoidea*. Mouth simple, surrounded by circlet of papillae; females enormously larger than males; viviparous (e.g. *Dracunculus*).

### 25.2 Type Example; *Rhabditis maupasi*

A number of the most readily obtainable nematodes belong to the genus *Rhabditis*. They occur in soil, water and decaying organic materials, and are facultative parasites

\* Anglicised terms in common usage.

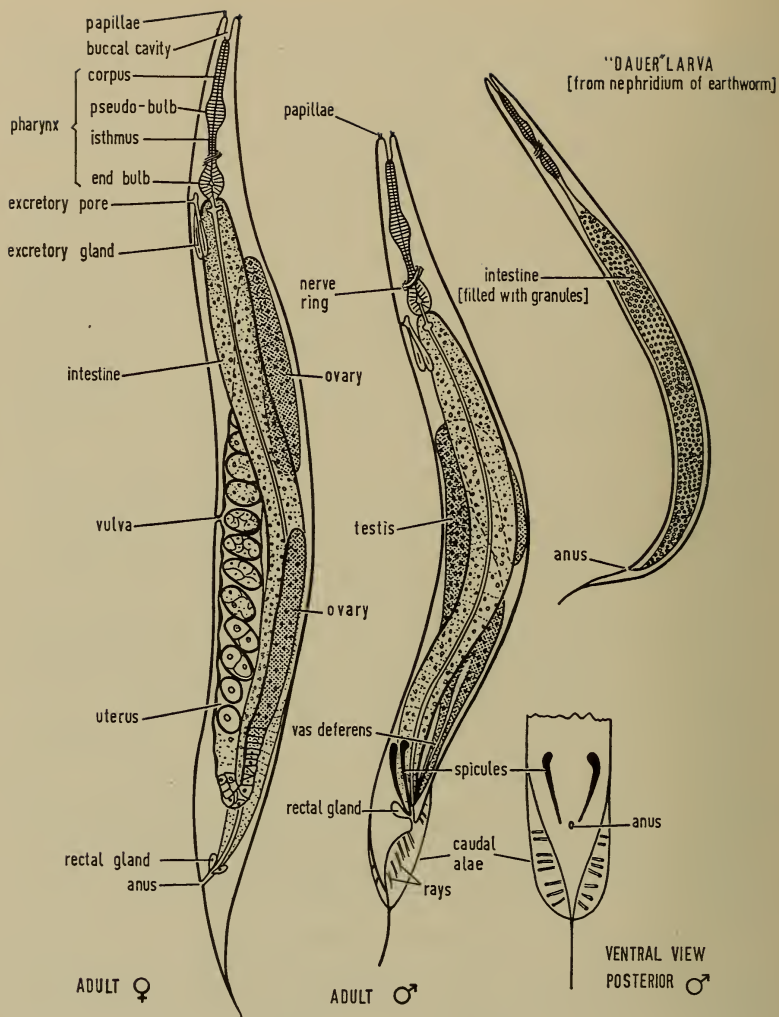


FIG. 117. *Rhabditis maupasi*—morphology of free-living males and females from laboratory culture (original).

in the larval stage. *R. maupasi* occurs commonly in earthworms. Other common species in the same host are *R. terrestris*, *R. pellio* and *R. anomala*, but it is difficult (even for those working on this group) to separate the species from the female or protandrous hermaphrodite. Males may be rare in some species. Young encysted forms (sometimes known as 'dauer' larvae although the term is used rather differently by various authors) occur in the ovoid brown bodies lying in the coelomic spaces of the posterior somites. Free forms may usually be found in the seminal vesicles, coelomic spaces and are especially common in the nephridia.

In the earthworm, rhabditids never develop beyond the juvenile stage, presumably due to the lack of certain growth factors, but with the available nutriment, are just able to survive without further differentiation. When an earthworm dies, however, the decaying flesh and the bacteria which thrive on it, provide additional food to raise the nutritional level to a degree which permits the juveniles to reach sexual maturity within a few days. Flourishing laboratory cultures are readily obtained by allowing a portion of the body wall of an earthworm containing nephridia, to undergo degeneration on nutrient agar plates (Fig. 118).

*External features.* Both sexes are cylindrical and elongated with a very thin, almost hair-like caudal termination or post-anal tail. The males (which in some 'races' are rare in laboratory cultures) are characterised by possessing a flared posterior end forming accessory copulatory organ (Fig. 117).

*Body wall.* A thin transparent cuticle covers the body. This is clearly secreted by the hypodermis, a subjacent layer containing a small number of nuclei. Beneath the hypodermis is a muscular layer consisting of a single sheet of longitudinal cells. *Rhabditis* is a meromyarian nematode (see p. 301), that is, it has few muscle cells in each sector. These muscle cells are unusual in that they have processes extending to the motor nerves instead of nerve processes extending to the muscles.

*Digestive system.* The intestine has a fine basement membrane supporting a single layer of cells. The pharynx and rectum have a cuticle, continuous with that on the exterior, which is shed at ecdysis. The mouth is enclosed in one dorsal and two ventral lips, each of which is further subdivided into two lobes, bearing a pair of sensory papillae. The narrow buccal cavity is heavily cuticularised and bears a ridge-like construction near its posterior end; the ridge bears fine cuticular teeth of systematic value. There is a muscular *pharynx* (=oesophagus) with a wide anterior region or *corpus* leading to a middle *pseudobulb*, connected by a narrow *isthmus* to a posterior *end bulb*, containing a tripartite valve; the bulb wall contains three gland cell nuclei. The arrangement of these cells, which ramify throughout the oesophagus, is of taxonomic importance.

**Excretory system.** The so-called 'excretory system' consists of a pair of lateral canals extending from the pharyngeal pro-corpus region nearly to the end of the worm, where they end blindly. These canals lead into an excretory sinus, which opens by a cuticular duct to the excretory pore. Also connected with the excretory sinus are a pair of sub-ventral cells. The excretory sinus bears a single nucleus; it is usually considered that the lateral canals are extensions of the sub-ventral cells (see p. 296).

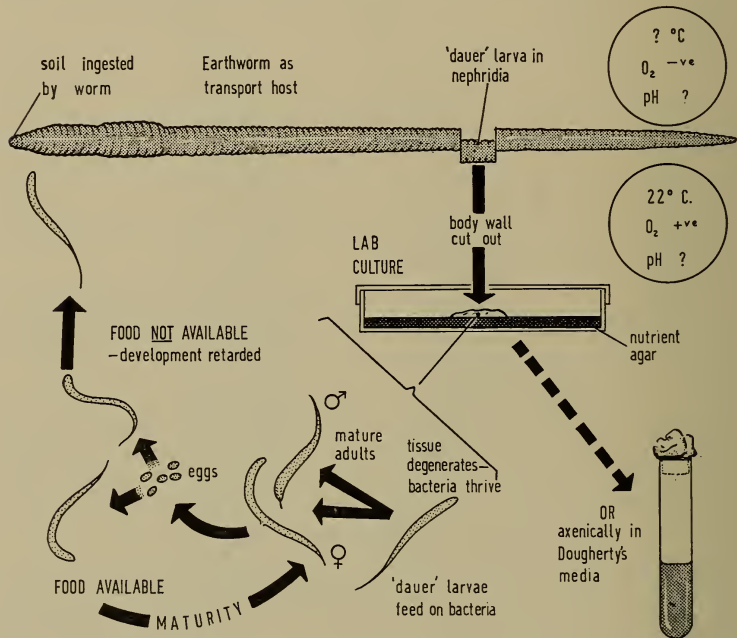


FIG. 118. Life cycle and laboratory culture of *Rhabditis maupasi* (original).

**Nervous system.** The general features of the nervous system are very similar in all nematodes. Only those parts easily seen in living unstained specimens will be described here. A nerve ring, composed chiefly of nerve fibres, surrounds the isthmus portion of the pharynx. Nerve cells lie in contact with the nerve ring and dorsal and ventral cords join it.



*Male reproductive system.* There is a single tubular testis reflexed at its anterior end and filled with spermatozoa in all stages of development. Spermatozoa are spherical and tail-less, a typical nematode feature. A well-developed vas deferens leads from the testis to the cloaca, in association with which are cuticular spicules with characteristic knobbed ends. These spicules appear to act as holdfast organs during copulation and are moved by protractor and retractor muscles. The tail of the male is further differentiated by being drawn out into longitudinal ridges—the *caudal alae*. These are thin-walled extensions of the postero-lateral body margins and also assist in holding the female in apposition during copulation. They are supported by nine pairs of *genital papillae*, finger-like projections of the ventral surface. Caudal alae are sometimes referred to as 'bursas', but this term, strictly speaking, is reserved for the wide caudal alae of the Strongylata (p. 326).

*Female reproductive system.* This is built on the typical nematode plan with paired ovaries converging on the vulva. The ovaries contain developing oocytes packed tightly together. The ends are retroflexed and each becomes constructed to form an oviduct in which spermatozoa may be found. Fertilisation takes place in this region, and eggs accumulate in the paired uteri, which connect by a short ventrally directed vagina, to the vulva.

In 'races' which are protandrous hermaphrodites, the worm has a genital system of the female form. Spermatozoa are first produced, then ova, which are fertilised by the stored spermatozoa.

*Life cycle.* This is shown diagrammatically in Fig. 118. When an earthworm dies, the contained third-stage 'dauer' larvae feed on the bacteria and fluid of the decaying organic material and develop into adults. These reproduce either bisexually or hermaphroditically and larvae produced from hatched eggs migrate into the soil to penetrate another earthworm via the nephridiopores or other apertures. Although the coelomic forms encyst, those in the nephridia and other sites remain free but, presumably due to the absence of suitable nutrients, they do not grow beyond the 'dauer' larva stage.

### 25.3 General Morphology of Nematodes

Nematodes are remarkable for exhibiting a high degree of uniformity in basic structural organisation. Their shape is essentially that of an elongated cylinder, inside which runs the body musculature entirely consisting of longitudinal fibres. Harris and Crofton (1957) have pointed out that a system of muscles of this kind can only operate against a suitable antagonistic force; since circular muscles are lacking, the return mechanism must be provided by alternative means such as internal hydrostatic pressure. They have shown that many of the morphological features of excretory, alimentary and reproductive systems can be correlated with this fact and the mechanical problems of

organisation which result. When nematode morphology is viewed in this light the result, discussed further on p. 303, is a most interesting integration of form and function. Although it is early yet to say whether these views will stand up to detailed examination when a number of species are examined, they may, if confirmed, revolutionise our ideas on nematode morphology.

In order to consider the morphology in the manner suggested by Harris and Crofton, the body wall, the musculature and the body cavity are dealt with later (p. 301), after the morphology of the other organ systems has been considered.

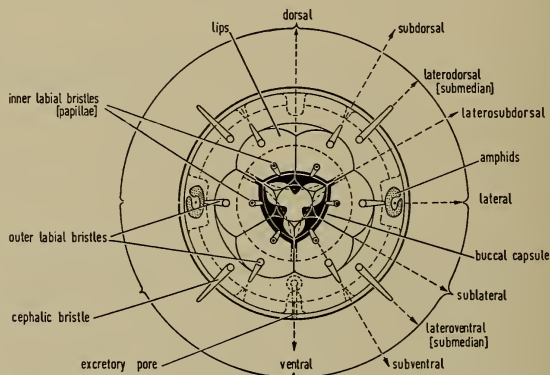


FIG. 119. Generalised diagram of end-on view of primitive nematode showing the hexamerous symmetry of the sensory bristles (modified from de Coninck, 1942).

### 25.31 External features

Recent studies have revealed that a marked hexamerous symmetry is superposed on the original bilateral symmetry of primitive nematodes. This hexamerous symmetry supports the view that nematodes may have evolved through a semi-sessile stage in which they were fastened posteriorly by caudal secretions to objects, and waved their bodies about in the water. Today many Aphasmodia can attach themselves by the secretions of the caudal glands.

The cephalic structures, which are of value for taxonomic purposes, are best studied from *en face* sections (Fig. 119). Primatively, the mouth is surrounded by six lips or labia each bearing a papilla, which may be modified to a bristle, so that a circle of six *inner labial papillae* is formed. Outside of this is an *outer labial circle* of six papillae, and outside of this again, in the cephalic region, is a *cephalic circle* of four papillae. The total number of anterior sense organs is thus considered to be sixteen, a condition found in

some free-living marine nematodes. In parasitic (and free-living, terrestrial) nematodes the number of anterior sense organs is usually greatly reduced and the two outer circlets reduced to one, so that at the most only two of the three circlets are present. In some species (e.g. *Ascaris*), the inner circlet is vestigial. Various other cuticular specialisations may be present in the head region.

**Special sense organs.** Under this heading are included two organs characteristic of nematodes and often of diagnostic value: *amphids* and *phasmids*. That they are, in fact, sense organs has yet to be demonstrated.

**Amphids.** Amphids are lateral cuticular excavations at the anterior end (Fig. 120). Into them open unicellular glands and they are supplied by nerves from the circumoral

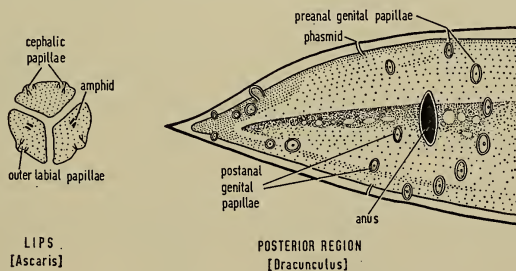


FIG. 120. Lips of *Ascaris lumbricoides* showing amphids, and tail of *Dracunculus medinensis* showing phasmids (after Thornton, 1924; Moorthy, 1937).

nerve ring. These are best developed in marine nematodes, but they are often inconspicuous in parasitic forms and so are of only limited taxonomic value.

**Phasmids.** Found in Phasmidia but not in the Aphasmedia as the names imply. They consist of a pair of posterior cuticular pouches, resembling amphids, and appear to be openings of unicellular glands; in some cases, they are reduced to mere papillae. Phasmids are better developed in parasitic (Fig. 120) than in free-living species in which they are usually absent.

### 25.32 Alimentary canal

**Buccal cavity.** This is variable in shape and size, and in degree of differentiation. In some forms, its cuticular lining may be moulded into rods or plates, often bearing teeth. The latter are particularly well developed in intestinal forms such as hookworms which rely on sucking blood from the mucous membrane (Fig. 145).

**Pharynx (Oesophagus).** The pharynx is one of the most characteristic features of nematode morphology. Although the term 'pharynx' strictly refers to a muscular oesophagus, the

terms 'pharynx' and 'oesophagus' as used in nematology may be considered to be synonymous.

The pharynx, which is provided with a triradiate lumen and radial muscle fibres, acts essentially as a muscular sucking organ. Its triradiate structure is continuous with that of the buccal capsule, lips and head sense organs. It is lined with cuticle, continuous with the exterior, and shed at ecdysis. The pharynx shows considerable variation, both in structure and mode of functioning, a diversity correlated both with widely differing feeding habits and with its phylogeny. Points of particular systematic value in the study of the pharynx (which is syncytial) are: the number and arrangement of cell nuclei, the form of the pharyngeal lumen and lining, and the form of the pharyngeal glands and the arrangement of the gland ducts. The pharyngeal glands are typically three in number, one dorsal and two ventro-lateral. They are long, branched structures and portions of them are met in almost any pharynx section. They are rather difficult to see in whole mounts because of the syncytial nature of the pharynx.

A complex terminology has grown up in relation to the nematode pharynx. The following definitions are based on Hyman (1951):

*bulb*: a muscular swelling containing sclerotinised valves capable of partly closing the lumen

*pseudobulb*: a muscular swelling lacking a valvular arrangement

*cylindrical*: of the same diameter throughout

*dorylaimoid*: slender anteriorly and wider posteriorly

*oxyuroid (bulboid)*: provided with an end bulb

*rhabditoid*: with a wide anterior region usually followed by a median pseudobulb, an isthmus and an end bulb

*ventricular (glandular) region*: a part of the pharynx devoid of muscle fibres

The form of the pharynx in the various sub-orders is as follows:

*Trichurata*. Markedly adapted for sucking. The wall, although muscular, is so reduced that it is virtually a capillary tube. A column of cells, the *stichosome*, lies along the oesophagus; the latter is sometimes embedded in it (Fig. 121).

*Dictyophymata*. Pharynx simple with no division into regions (Fig. 121).

*Rhabditata*. Usually possess the 'rhabditoid' type of pharynx, characteristically with an anterior pseudobulb and a posterior valved bulb (Figs. 117, 121).

*Ascaridata*. Form of the oesophagus varies more than any other group. In the Oxyuroidea, it is basically of the rhabditoid type. In the Ascaridoidea, the pharynx is usually cylindrical and lacks a bulbar enlargement. Posterior pharyngeal diverticula, known as appendices or intestinal pouches, are not uncommon. Larval ascarids may show traces of a rhabditoid pharynx in their early larval stages (Fig. 121).

*Strongylata*. Oesophagus simple with no differentiation into regions (Fig. 121).

*Spirurata*. Pharynx cylindrical and divided into an anterior muscular part and a posterior wholly glandular portion (Fig. 121).

*Camallanata*. Pharynx usually divisible into a narrow anterior muscular portion and a broader posterior glandular portion (Fig. 121).

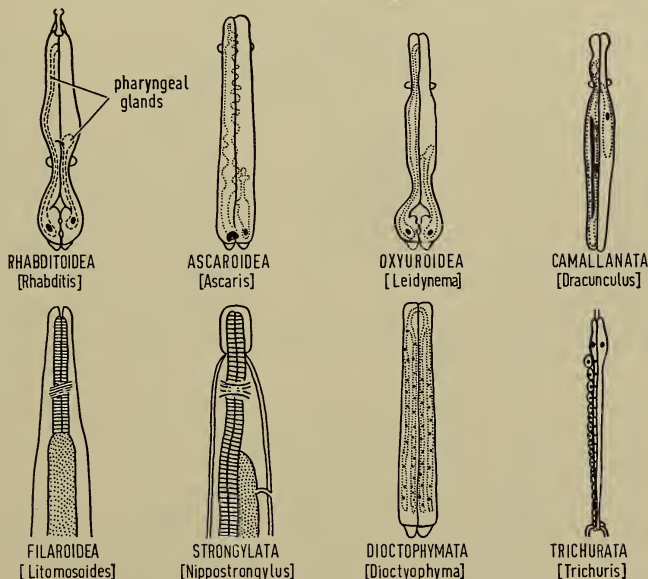


FIG. 121. Form of pharynx in the various groups of nematodes (after a number of authors).

*Intestine*. The morphology of the intestine does not differ markedly in the various sub-orders. In general, it is a straight tube showing little differentiation into regions: an anterior or ventricular region, a mid-region or intestine proper and a posterior or prerectal region. The differences between these regions are mainly reflected in the cell height of the epithelial lining and the shape of the lumen. There may also be some difference in the type of cell inclusions.

*Layers*. The wall of the intestine (Fig. 122) is made up of a single layer of epithelial cells which bear on their internal surface a border of fine hair-like structures somewhat



resembling the brush border of vertebrates. This is the *bacillary layer* (*bordeur en brousse*, *Stabchensaum*) whose appearance varies somewhat with fixation.

Several views have been put forward to explain its structure and possible function. Thus it may be a layer of degenerate or amalgamated cilia, a secretory product of a protective nature, or a development of fine tubes which act in excretion or absorption. This layer is not peculiar to nematodes among the invertebrates, as a similar layer occurs in annelids as well as in arthropods.

*Cell inclusions.* As in most invertebrate animals, the gut cells contain a number of inclusions which are either food reserves or waste products. Glycogen, neutral fats and

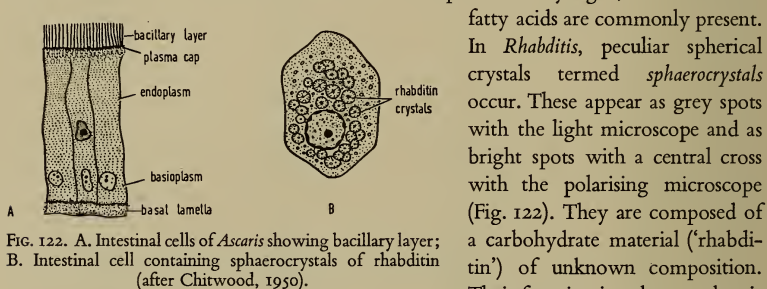


FIG. 122. A. Intestinal cells of *Ascaris* showing bacillary layer; B. Intestinal cell containing sphaerocrystals of rhabditin (after Chitwood, 1950).

fatty acids are commonly present. In *Rhabditis*, peculiar spherical crystals termed *sphaerocrystals* occur. These appear as grey spots with the light microscope and as bright spots with a central cross with the polarising microscope (Fig. 122). They are composed of a carbohydrate material ('rhabditin') of unknown composition.

Their function is unknown, but is probably related to the nutritional habits, for the crystalloids appear when the larvae become 'dauer' larvae, or when worms are cultured in rich medium. When a culture is becoming exhausted they do not appear at all.

*Rectum.* The hind gut is lined with cuticle continuous with the external cuticle. In the female it becomes a rectum, but in the male the reproductive system always opens into it so that it becomes a true cloaca. A number of unicellular rectal glands (three to six) open into the rectum in the majority of species (except in the Aphasmidia). *Nervous system.* In all forms studied, the structure of the nervous system is remarkably constant. The main part of the nervous system consists of a ring around the pharynx with a number of associated ganglia. The largest of these are paired lateral ganglia (Fig. 123) and a single or paired ventral ganglia. Other smaller ganglia are present. From the nerve ring the ganglia give off backwards: (a) a mid-dorsal nerve, (b) a mid-ventral nerve and (c) one to three pairs of lateral nerves. The mid-dorsal and mid-ventral nerves are motor nerves associated with muscular contraction. Anteriorly from the nerve ring pass six nerves to the head sense organs.

*Excretory system.* The excretory system is perhaps the most varied anatomical feature of nematodes. It possesses no flame cells, protonephridia or other current-producing



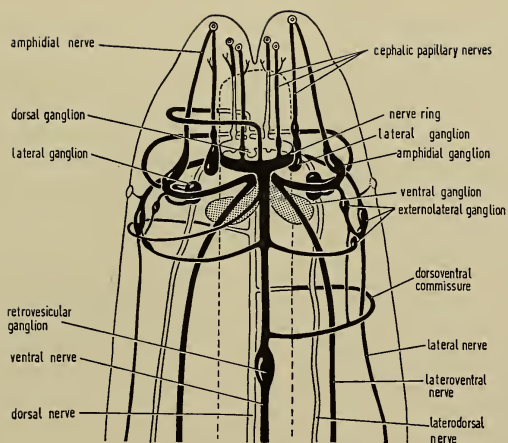


FIG. 123. Anterior part of nervous system of *Ascaris lumbricoides* (after Goldschmidt, 1908).

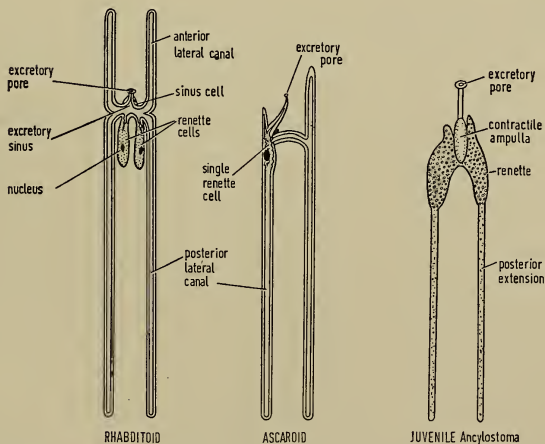


FIG. 124. Nematode excretory systems (after Chitwood, 1950; Stekhoven, 1927).

mechanisms. The structures to which an excretory function has been ascribed are of two sorts, glandular and tubular.

*Glandular type.* This is best developed in marine nematodes which are generally considered the most primitive of living nematodes. It consists essentially of one or two gland cells situated in the posterior ventral region of the pharynx and opening via a long neck to the excretory pore (Fig. 124); the whole is termed a *renette*.

*Tubular system.* This is related to the glandular system and probably developed from it. The renette is often poorly developed in the adult stages although well formed in the

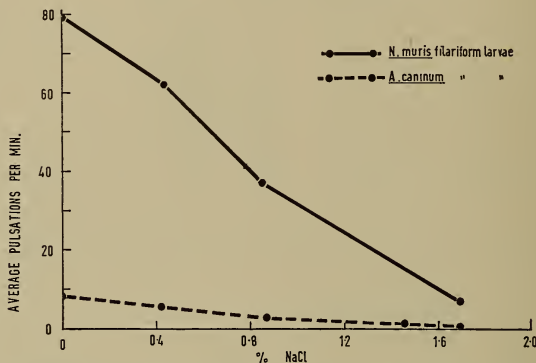


FIG. 125. Pulsation rate of the excretory vesicle in filariform larvae of *Nippostrongylus muris* and *Ancylostoma caninum* in solutions of different osmotic pressure (redrawn from Weinstein, 1952).

larval stages. Thus, in young *Ancylostoma*, the renette cells are drawn out into tubular extensions and it is tempting to assume that they give rise to the adult tubular excretory system. The canal system is most frequently H-shaped, but the anterior limbs, or one side of the system may atrophy giving an asymmetrical arrangement (Fig. 124).

In larval nematodes, the canal system may pulsate rhythmically and in some species it has been shown to play a major part in the maintenance of water balance, thus serving the same function as the contractile vacuole in protozoans. The rate of pulsation varies inversely with the salt concentration in the medium (Fig. 125).

## 25.4 Reproduction

Most nematodes are dioecious, but protandrous hermaphrodites and parthenogenetic females are not uncommon. Experimental changes in the environment have

been used to induce changes in the sexual characteristics of individuals, and even complete sex reversal has been achieved by this means. The chromosome changes in such cases have been studied in detail by Nigon (1949). Males are usually distinguishable from females by their smaller size, posterior curvature and presence of accessory copulatory structures such as spicules, bursae or genital papillae.

*Male system.* The male system was undoubtedly originally double, but is single in the majority of Phasmodia. The male system of *R. maupasi*, already described (p. 289), is fully representative of the nematodes.

With a few exceptions (e.g. *Trichinella spiralis*, p. 307), males possess *copulatory spicules* secreted by and lodged in cloacal evaginations termed *spicule pouches*. There are usually two such pouches which unite before entering the cloaca. Spicules, which are hard sclerotinised cuticular structures, are typically two in number, but occasionally single. They show great variations in size and shape. The spicule pouch may be thickened dorsally to form an accessory piece or plate, the *gubernaculum*, which serves to guide the spicules (Fig. 126). A ventral thickening of the ventral and lateral cloacal wall, termed a *telamon*, may also occur; these terms are often confused in the literature.

In nematodes of the order *Strongylata*, the cuticle in the posterior region is expanded into a structure, supported by ribs or rays, termed a *bursa* (Fig. 145), and species possessing this structure are said to be 'bursate'. Many authors restrict the term 'bursa' to the structure found in the male strongyloid, but the term is also loosely used to describe any similar posterior male expansion such as the cuticular alae in the rhabditoids and the bell-like muscular organ of the Dioctophymata.

The permatozoa of nematodes are unusual in being round, conical or elongated bodies, which, even in the case of those with an apparent tail, move by an amoeboid movement.

*Female system.* There are usually two ovaries; rarely one or more than two. They are generally orientated in opposite directions and may be straight, reflexed or, when long, wound backwards and forwards. The ovary is made up of a continuous sac consisting of an epithelial layer and a *germinal cord*. The epithelial lining consists of a single layer of squamous epithelium which in the oviduct region becomes transformed into high columnar epithelium. The germinal cord contains the maturing oogonia which often lie round a central strand or rachis whose functions are uncertain. The epithelium of the ovary continues distally as the oviduct with characteristic columnar epithelium. This, in turn, passes into the wider uterus, made up mainly of squamous epithelium. Sperms are stored in the lower part of the uterus where fertilisation occurs. The uteri lead into a

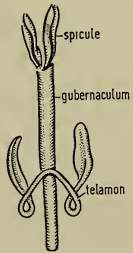


FIG. 126. Male armature of a trichostrongyloid, with telamon (after Hall, 1921).

short muscular vagina which opens by the vulva, usually situated about one-third the body distance from the anterior end. There is sometimes an undivided uterine part between the uterine tubes and the vagina. In many strongyloids and spiruroids, the posterior end of the female may become heavily muscularised to form an ovejector for the expulsion of eggs.

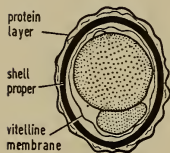


FIG. 127. Egg of *Ascaris lumbricoides* (after Chitwood, 1950).

*The nematode egg shell.* The egg shell is particularly well developed in many parasitic nematodes and serves as a highly protective mechanism for that stage of the life cycle. It is typically oval or round and is made up of three coverings (Fig. 127):

- (a) an external protein coat (secreted by the uterine wall);
- (b) a middle chitinous membrane or shell proper (secreted by the egg);
- (c) an inner vitelline lipoidal membrane (secreted by the egg).

(a) *Protein coat.* This layer is often sculptured, mammillated (i.e. covered with protuberances) or drawn into filaments. It consists of a fibrin-like or an elastin-like structural protein or both. The protein is probably always subjected to quinone tanning and in some cases, to keratinisation (Monné, 1955). The polar plugs found in some nematode eggs (Fig. 130) consist of polysaccharide (Monné and Hönig, 1954).

(b) *Shell proper.* This appears to consist of an outer protein layer and an inner layer of chitin or a substance closely related to chitin. During the early stages of development, the ovocytes secrete glycogen globules and lipid globules. The former move to the surface where they break down into glucose from which glucosamine, the chief constituent of chitin, is synthesised. The lipid globules move to the surface and form the lipid membrane.

(c) *Lipoid (vitelline) membrane.* Varies considerably in different species, from very thick to very delicate; it serves as an effective barrier to many chemicals. In *Ascaris* it is reported to contain 75 per cent ascarosides and 25 per cent protein.

*Egg-laying habits.* The majority of parasitic nematodes are oviparous, the eggs being discharged outside the female body. Some are *ovoviviparous*, in which case the eggs hatch while still within the uterus. This is a widespread habit in parasitic forms. Many of the so-called viviparous species are actually ovoviviparous, and it is questionable whether viviparity in the true sense of the word ever occurs.

The so-called microfilariae of the Filarioidea (p. 342) are enclosed in an elastic 'sheath' (Fig. 149) which takes on the contours of the larva. It is thought that this 'sheath', in fact, represents the modified egg shell in these forms, although there is some controversy on this point.

*Development.* The embryology has been the subject of several classical studies in cytology and cell lineage. Development is highly determinant in character and the fate of each cell can be definitely followed. It is remarkable for two reasons (a) the separation of the germ cell line after the first cell cleavage, and (b) the absence (usually) of somatic cell

multiplication after hatching. As there is relatively enormous size difference between larva and adult, it follows that an unusual amount of cytoplasmic growth occurs, resulting in the production of monster cells. In the larger nematodes, for instance, muscle cells may be several millimetres long. There is also a marked tendency for nematode tissues to form syncytia.

The process of growth in size of nematodes, like that of arthropods, is accompanied by ecdysis. As far as is known, there are always four moults and the nematode formed as the fifth stage is the adult, although some growth in size may follow. The stages in the post-embryonic growth of nematodes are usually referred to as 'larvae', although according to some authors (notably Hyman) the term 'juveniles' is a more suitable term for such stages.

The terminology of larval development is straightforward. After the first moult, larvae are termed second-stage larvae; after the second, third-stage, and so on. The stage at which the larvae are capable of living in the host is termed the *infective* stage. In some species, two moults take place within the egg, so that two cuticles are present when the larvae hatch. In some cases, the cast sheath is retained by the larva as a loose-fitting coat and this probably has some protective function.

The degree of development of the egg when laid varies considerably with species. In some, the eggs are laid in an unsegmented condition (e.g. *Ascaris* and *Trichuris*), while in others, early segmentation has proceeded (e.g. hookworms). Still others (e.g. *Syphacia*, Fig. 141) may reach a stage with a coiled larva visible, and in the ovoviparous species and the so-called viviparous species, fully developed larvae are released by the females (e.g. *Dracunculus*).

*Life cycles.* The life cycle of different species shows interesting variations on the theme egg-larvae-adult. Although almost every organ of the body has been used as a nematode habitat, the best-known species are intestinal parasites with a direct life cycle not involving an intermediate host. In the simplest type of life cycle (e.g. *Enterobius*), embryonated eggs are swallowed by the host and hatch in the intestine where they reach maturity. Some directly developing species (e.g. *Ascaris*), after hatching in the intestine, undergo a curious and unaccountable tissue migration through the body before returning to become established in the intestine. The simple type of life cycle represented by *Ascaris* may be varied in other species by the first-stage embryo hatching outside the host body, developing to an infective larva, and reinfecting its host by skin penetration or oral entry.

In the indirect type of life history, use is made of invertebrate intermediate hosts, insects (usually beetles and cockroaches), crustaceans (especially copepods) and molluscs

(snails and slugs). In one group (the Mermithoidea) the larval stage is a parasite of an invertebrate and the adult free-living. Detailed accounts of individual life cycles will be dealt with later.

In the alternative type of life history, there may be an alternation of a parasitic generation with a free-living one. The best known are those of various species of the genus *Strongyloides*, in which larvae may develop to free-living adults if the external nutritional conditions are suitable.

Some genera (e.g. *Neoaplectana*, p. 317) are facultative parasites. They may pass a number of generations in dead insects, but when food is exhausted, resting larval stages remain capable of invading living insects as parasites.

### 25.5 Body wall

The body wall of nematodes consists of: (a) cuticle, (b) epicuticle (hypodermis, sub-cuticle), and (c) muscle layers (Fig. 137).

*Cuticle.* This consists of a number of layers which have a complex histological and chemical composition. In *Ascaris lumbricoides*, there are nine layers which fall roughly into three groups as follows:

Cortex	{ outer cortical inner cortical
Matrix or homogeneous stratum	{ fibrillar homogeneous boundary
Fibrous layers	{ outer fibre middle fibre inner fibre basal lamella

However, *Oxyuris equi* and *Strongylus equinus* have only eight layers (Bird, 1958).

Unlike the cuticle of insects, the nematode cuticle contains no chitin. Although most workers quote the fibre layers as being made of collagen, electron microscopy of the fibre layers, and of the basal lamella, has revealed that no typical collagen periodicities are present in either of the structures (Bird and Deutsch, 1958). The external coating is made up of two proteins, a fibrin-like protein and elastin-like protein, and these are partly subjected to quinone-tanning and probably also to keratinisation (Monné, 1955; Bird, 1957).



In certain plant nematodes, the 'eelworms', the mature female becomes rounded and cyst-like, the cuticle acting as a cyst wall which hardens by quinone tanning. Analysis of the cuticle shows the presence of albumins, glycoproteins and proteins termed matricin, ascarocollagen and keratin, which correspond to the matrix, fibre and external cortical layers respectively.

Little is known regarding the permeability of the nematode cuticle or the role it may play in permitting absorption or excretion to take place.

In *Ascaris*, and probably most nematodes, the fibres of the three fibrous layers cross each other diagonally, and are inclined at about  $70^\circ$  to the long axis of the worm. Thus any two sets of fibres enclose a system of minute parallelograms (Fig. 129). On the 'lazy tongs' system, these parallelograms may be distorted by shearing, with a consequent change in the length of their diagonals. In relation to the structure of the nematode, this will lead to the worm becoming longer and thinner or shorter and thicker. The cuticle is thus not theoretically inelastic as usually believed, and Harris and Crofton (1958) found experimentally that half worms artificially inflated showed changes in length of the order of 10-15 per cent. They further showed that there was a clear correlation between tail shortening and an increase of internal pressure. Thus, when muscles contracted in the tail region, they exerted an additional pressure on the pseudocoelomic fluid (see below). This produced a volume transfer to the head region with an increase in head pressure and subsequent head lengthening.

*The epicuticle.* Often known as the sub-cuticle or hypodermis. Consists essentially of a thin syncytial layer, beneath the cuticle, which bulges into the pseudocoel to form four longitudinal ridges or longitudinal cords. The lateral cords are usually the most prominent of these four lines, and may be seen on the surface as pale lines.

*Musculature.* The body wall musculature consists exclusively of longitudinal fibres. The form, number and arrangement of muscle cells varies greatly with species and the following terminology is used to describe them.

(a) *Holomyarian.* In which there appear to be no definite muscle cells, the muscle layers being either continuous or divided into zones by the lateral cords.

(b) *Meromyarian.* In which there are only a few rows of muscle cells, about three to five in each longitudinal strip (Fig. 128).

(c) *Polymyarian.* In which there are large numbers of rows of muscle cells in each longitudinal strip (Fig. 128).

These terms may not always be precisely applicable since transitions occur between these various conditions.

The cytology of the nematode muscle cell is of especial interest, for each muscle fibre is only one enormously elongated cell. These cells have *fibrillated* and *protoplasmic* zones. The fibrillated zone consists of longitudinal bands of homogeneous contractile substance alternating with material which is non-

contractile and which contains supporting fibrils. The protoplasmic part may contain some supporting fibrils. In some species, the muscle cells are flat with fibrils confined to the region parallel to the hypodermis, a condition known as *platymyarian* (Fig. 128). In others, the protoplasmic zone bulges into the pseudocoel with the fibrillated zone extending along the side; this condition is termed *coelomyarian*. This is usually clearly seen in sections of *Ascaris*.

It has been pointed out (p. 301) that circular muscles are lacking. The longitudinal muscles must operate against some antagonistic force which, according to Harris and Crofton (1957) is provided by the internal hydrostatic pressure of the pseudocoelomic fluid.

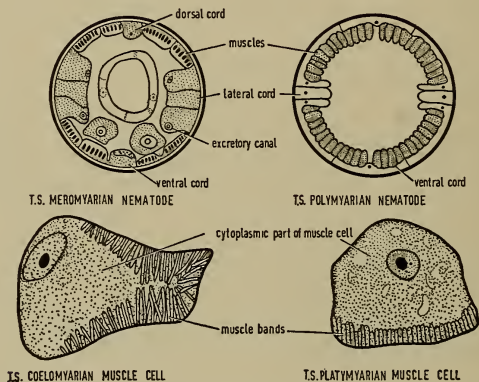


FIG. 128. Types of muscle-cell organisation in nematodes (after various authors).

It is also pointed out (Harris and Crofton, 1958) that, as a consequence of the lateral cords dividing the muscles into dorsal and ventral regions, nematodes bend dorso-ventrally and not laterally as do fishes and annelids.

*Pseudocoel*. The body cavity between the body wall and viscera contains a fluid which, in *Ascaris lumbricoides*, has a mean hydrostatic pressure of 70 mm Hg (95 cm of water) with a range of 16–225 mm (Harris and Crofton, 1957). This fluid, which has a freezing point depression of  $\Delta = -0.65^{\circ}\text{C.}$ , and a pH of 6.8, contains about 6.8 per cent dry matter. The pseudocoel also usually contains fibrous tissue and fixed cells or nuclei. The fixed cells (*pseudocoelocytes* or *coelomocytes*) vary in number from two to six, but occupy fixed positions usually in relation to the longitudinal cords. These cells are often

highly branched or stellate and are associated with fibrous strands or membranes which constitute a kind of mesenchyme. Their function is uncertain but may be related to the secretion of oxidative enzymes.

### 25.6 General Considerations of Structure and Function

It has been pointed out (p. 301) that, in the absence of circular muscles, the longitudinal muscles act against forces exerted by the internal fluid on the body wall. According to the views of Harris and Crofton (1957, 1958), many of the morphological features of nematodes can be interpreted as representing adaptations to a changing body length and a high internal pressure.

Thus, the alimentary canal is thin-walled, flexible and capable of extension. The presence of dilator muscles (the *depressor ani*) would be correlated with a high internal pressure; without such a mechanism the gut contents would be forced out. The triradiate pharynx could act as a valve anteriorly and also serve to pump food into the gut, against the internal pressure.

The excretory tubes embedded in lateral cords would be well adapted to resist collapse under pressure. In the reproductive system there is present a peristaltic musculature and true sphincter muscles. This allows for controlled emission of eggs under pressure by a combination of peristaltic movement (which pinches off the selected group of eggs) and a sphincter muscle which releases the eggs at the appropriate time. The absence of flagella in the sperms does not appear to be adapted to any of the mechanical requirements mentioned above, but may be connected with the general absence of cilia.

These views are based on observations on *Ascaris lumbricoides* and it is difficult to

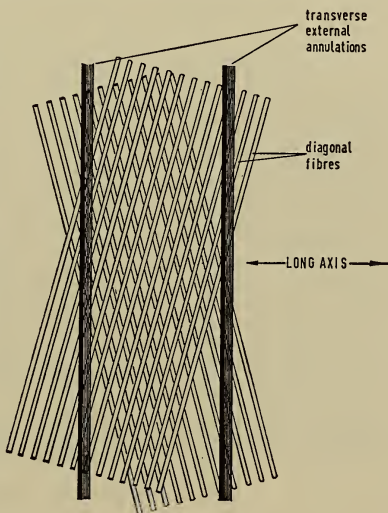


FIG. 129. A schematic diagram illustrating the orientation of the two fibril layers in the cuticle of *Ascaris lumbricoides* in relation to two of the transverse external annulations (adapted from Harris and Crofton, 1957).

say, at this stage, whether from specialised studies a generalisation regarding the determining factors of nematode morphology can be made. It is likely that this will prove to be the case, for on theoretical grounds it can be argued that 'the mechanical factors which determine the general features of the nematode will be independent of the scale on which the model is built' (Harris and Crofton, 1957).

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## CHAPTER XXVI

# NEMATODA: APHASMIDIA

### 26.I Sub-order I. Trichurata

Worms of this sub-order are characterised by the possession of an enormously elongated capillary-like oesophagus, whose internal cavity is so small that it is difficult even to locate in sections (Fig. 131). The oesophagus is embedded in a long column of single cells which may serve as oesophageal glands, the whole structure being referred to as a *stichosome*. The anus is at the extreme posterior tip, and not just in front of the hind end, so that there is no tail. The male has one spicule or none at all; if present, it is enclosed in a protrusible membranous sheath.

The three families are diagnosed as follows:

*Trichuridae*. The 'whip worms'. Oviparous. Adults in intestine. Males with protrusible spiny sheath and spicule (e.g. *Trichuris trichiura*).

*Trichinellidae*. Viviparous. Male with no spicule or spicule sheath. Larvae in tissue; adult in intestine (only *Trichinella spiralis*).

*Trichosomoididae*. Ovoviviparous. Adults in urinary bladder of rodents. Male minute and parasitic in vagina of female worm (e.g. *Trichosomoides crassicauda*).

### 26.II Family Trichuridae

Genus: *Trichuris*

These are the so-called 'whip worms', a term derived from the whip-like form of the body. The characters of the genus are discussed by Chandler (1930).

The correct generic name for members of this group is *Trichuris*—a term which gives misleading description, for it literally means 'thread tail'. The name was applied before it was realised that the thin thread-like part which looked like the tail was, in fact, the head. A more apt name, then, is *Trichocephalus*, which was later applied, but the earlier name *Trichuris* has zoological priority.

The best known species are as follows:

*Trichuris muris*: rodents.

*T. ovis*: sheep, goats, cattle and other herbivores.

*T. trichiura*: man, pigs and monkeys.



*General morphology.* This does not vary much. The thread-like oesophageal region occupies about two-thirds the length of the body. The mouth has no lips. The vulva is at the junction of the thread-like and thickened regions of the body. The eggs have a characteristic barrel shape with an opercular plug at each end (Fig. 130).

*Life cycle.* The life cycle is similar in all species. Eggs require a warm moist environment for embryonation (such as that provided by rain-soaked soil), but once embryonated are exceptionally resistant to environmental conditions. Embryonated eggs hatch near the caecum into which they later migrate. Possibly related to scarcity of food materials

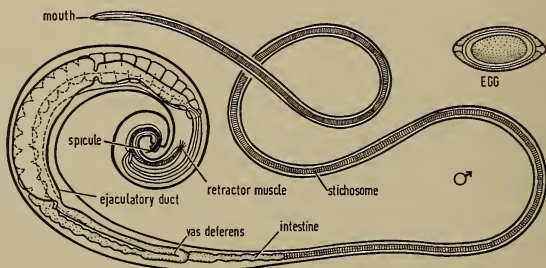


FIG. 130. *Trichuris trichura*—morphology of male and egg (after Brown, 1950).

in this site, development is slow, and 70–90 days are required for maturation. Heavy infections in humans cause abdominal discomfort, anaemia, and eosinophilia.

As the eggs of both *Ascaris* (p. 320) and *Trichuris* require the same conditions for embryonation (high humidity and access to oxygen) these two species frequently occur together in humans.

#### Genus: *Capillaria*

In the worms of this genus, the characteristic division into a narrow anterior region and a broader posterior region is not so marked. They are parasitic in a number of warm-blooded animals particularly birds and small carnivorous mammals.

*Life history.* The life history varies rather unusually with species. In *C. columbae* (pigeons) and *C. aerophila* (foxes) development is similar to *Trichuris* with direct embryonation of the eggs outside the host and reinfection by swallowing embryonated eggs. In *C. hepatica* (rodents), the adults occur in the liver and released eggs accumulate on the surface of the liver. As the eggs require oxygen for normal embryonation, ingestion of



an infected liver by another animal will not lead to an infection, but eggs subsequently passed undigested into the faeces, when exposed to air and moisture, become infective on re-ingestion.

The capillarid worms *C. longicollis* and *C. annulata* make use of earthworms as intermediate hosts. Embryonated eggs are not infective if swallowed directly by the definitive host, but if ingested by an earthworm become infective within twenty-four hours. The larvae are released within the earthworm and enter the connective tissue of the body cavity; the bird hosts become infected by eating earthworms.

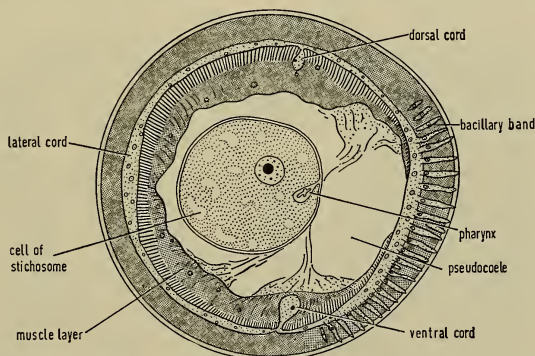


FIG. 131. *Trichuris trichiura*—section through pharyngeal region, showing the narrow pharynx embedded in the stichosome (after Ranther, 1918).

## 26.12 Family Trichinellidae

This family contains only the species *Trichinella spiralis*, the 'trichina', which is entirely a parasite of carnivorous animals. It has a widespread distribution, but, unlike most parasites of man, is confined to cold or temperate climates. It shows a wide host spectrum, and although its most common hosts are undoubtedly man, pig and rat, it occurs also in polar bears, cats, dogs, seals, walruses and whales.

**Morphology.** Both sexes are small, the male (1.4–1.6 mm) being smaller and more slender than the female (3–4 mm). Spicules are absent in the male, but the cloaca is everted during copulation and its opening flanked by two papillae. The female genital system is single (Fig. 132).

**Life history.** Infection is brought about by a host eating meat containing encapsulated larvae. These larvae are released in the duodenum and may be found there as early as one hour after the initial infection. Within four hours many become lodged in the

intestinal villi and penetrate deep into the glandular tissue and even reach the muscularis mucosae. Larvae mature exceptionally rapidly and within twenty-four hours after ingestion, copulation occurs. The males die soon after copulation. The females are ovoviviparous and up to 1,500 larvae are produced by a single worm. Released larvae reach the bloodstream, usually via the hepatic portal vein, but probably by other routes also, and are carried all over the body. Some of these tissue sites may represent environments unfavourable for growth and larvae will fail to become established. Other tissues,

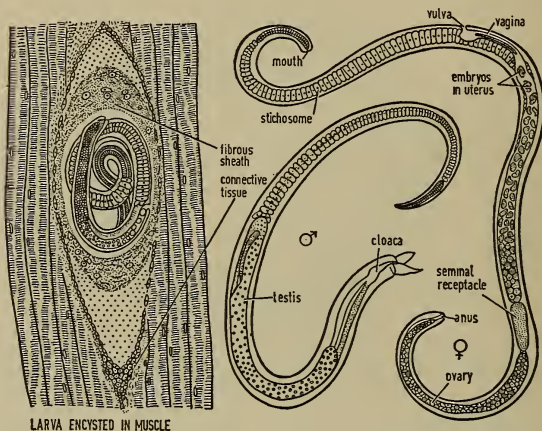


FIG. 132. *Trichinella spiralis*—morphology of male and female and encysted larvae in muscle fibres (after Brown, 1950).

particularly voluntary muscles, and especially those of the jaws, tongue, diaphragm, larynx and eye, provide suitable environmental conditions. Larvae actually penetrate into the muscle fibres (Fig. 132) ultimately causing degeneration of the fibre and thickening of the sarcolemma. The muscle cells react in a typical manner and undergo a 'host-tissue reaction' (p. 366) resulting in the formation of a connective-tissue capsule or cyst, the shape of which varies from round to oval in different hosts.

About six months after formation, the cysts begin to calcify and the process continues until the complete cyst wall is calcified. Larvae may remain viable for several years within the cysts but they never develop further. Even at this stage, however, considerable sexual differentiation takes place and male and female primordia are well

developed. Thus when the infected muscles are eaten by the second host, both males and females are able to mature within 24–72 hours and to undergo copulation. Complete sexual maturity never takes place within the cysts, probably due to lack of suitable food supplies.

The normal life cycle in the British Isles, the United States and Canada is through the series of hosts: rat–pig–man–pig (see Fig. 133). The rat is probably the most highly infected ‘natural’ host and pigs become infected by eating infected pork scraps or occasionally rats which penetrate into their stalls. Man becomes infected by eating imperfectly cooked pork and, as mentioned, may reinfect pigs by feeding back scraps of infected pork in pig food. Sausages are a dangerous source of the parasite, as a small piece of pork after mincing may be distributed amongst a number of sausages, although as factories pool pork from many animals considerable dilution may take place.

There is evidence, at least in Russia, and possibly in other countries, that the larva of necrophagous beetles and other insects, which form part of the food of some carnivores, can act as short-term reservoir hosts. Such hosts could serve to transfer infections from carcasses to carnivores, omnivores and insectivores. The period of survival in insect larvae is only about six to eight days (Merkushev, 1955).

It is estimated that some 16 per cent of all the inhabitants of the United States are infected with *Trichinella* to a greater or lesser degree, but the figures in the British Isles are less. Such figures are arrived at from autopsy reports based on an examination of diaphragms. There seems no doubt that the majority of light trichina infections are overlooked. In one particular survey, over 200 postmortem survey infections were detected and yet trichinosis had not been diagnosed or even suspected in a single one of these cases. Accurate diagnosis presents an interesting biological problem in both man and animals. Faecal examination for adults is unreliable. A skin test (Bachman) and a flocculation test (Bozicevich) are available, the latter being the most reliable. The disease is termed *trichinosis*.

*Immunity.* Animals, including man, rapidly develop an immunity to *Trichinella* (see p. 390). Antigens have been found in the blood as early as twenty-four hours after the initial infection. Although complete immunity is not found, a high degree of protection is developed, and worms in immunised hosts show retarded development and inhibited reproduction. Some small degree of protection may also be obtained from injections of vaccines or sera. The excretions and secretions of *Trichinella spiralis* may have an antigenic role (Campbell, 1954).

*Effect of temperature.* The range of temperatures within which the encysted larvae will remain viable has an important bearing on the biological control of the disease. A temperature of 55° C. rapidly kills the larvae, whereas exposure to cold (about –15° C.) for forty-eight hours, while not killing the larvae, renders them non-infective. The reasons for this are not understood. The U.S. Bureau of Animal Industry makes use of this knowledge and requires uncooked pork to hang at –15° C. for twenty days before distribution.

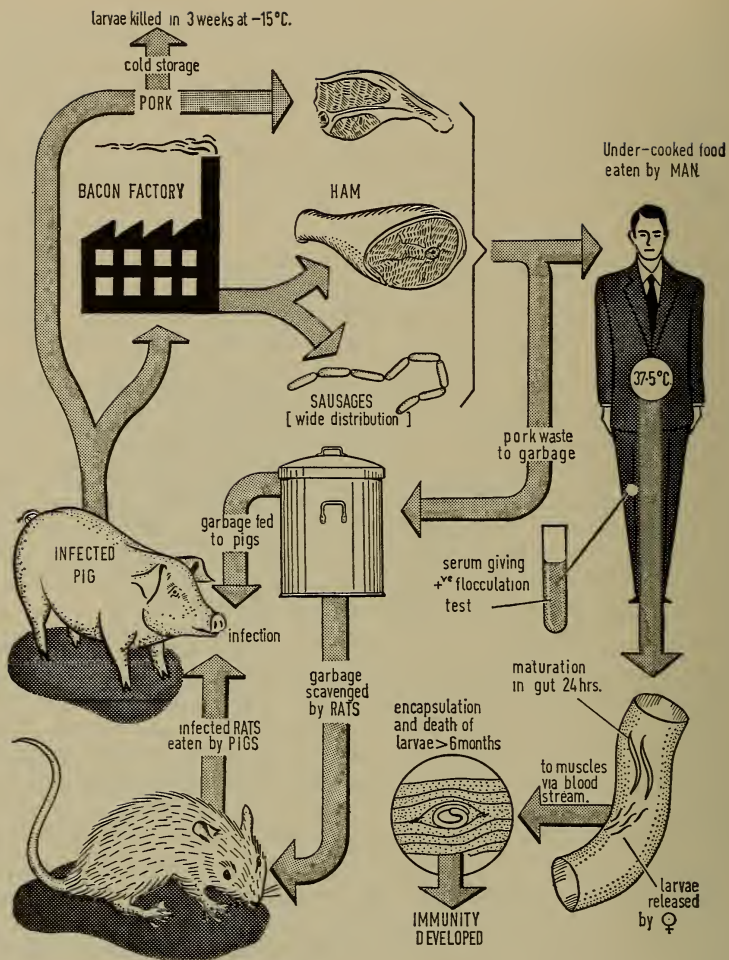


FIG. 133. *Trichinella spiralis*—life cycle and probable mode of transmission in European and American communities (original).



### 26.13 Family Trichosomoididae

This is an interesting family about which little is known. *Trichosomoides crassicauda* lives in the bladder, kidney pelvis and ureters of rats. Its life cycle has not been worked out in detail. The eggs hatch in the stomach and released larvae pass to the lungs where they are probably carried by the blood stream to the bladder. This species is remarkable for the fact that the male not only lives in the uterus of the female but is hyperparasitic there, three or four males occurring within a single female.

### 26.2 Sub-order 2. Dioctophymata

Members of this sub-order are large forms parasitic in the kidneys or occasionally the peritoneal cavity of mammals, including man. Dogs, foxes, otters, minks and other fish-eating carnivores have all been reported as hosts. The example chosen has a widespread distribution, but is rare in the British Isles. In some countries, especially Canada, it may be of economic importance as a parasite of mink.

#### *Dioctophyma renalis* (*Dioctophyma renale*)

*Morphology.* This is one of the largest nematodes known, the female being up to 103 cm long with a diameter of 5–12 mm; the male is considerably smaller (35 cm  $\times$  3–4 mm). The female has one ovary, an anterior vulva and a terminal anus; the male has a terminal bell-shaped bursa with a spicule 5–6 mm long. The worms are blood-red in colour.

*Life cycle.* The life cycle is very imperfectly known. In the case of the parasite found in wild mink, the cycle involves an annelid, a crayfish and a fish. Infective eggs hatch when swallowed by the branchiobdellid oligochaete *Cambarinocola chirocephala* commensal on crayfish and the first larvae encyst in the tissues of the crayfish. When infected crayfish are eaten by fish the larvae pass through the third and fourth larval stages. Mink becomes infected by eating infected fish, and the larvae make their way to the kidney or more rarely to the peritoneal cavity. Whole or part of the kidney may be slowly digested away, often only leaving the capsule of the kidney as a bag enclosing the worms.

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## CHAPTER XXVII

# PHASMID NEMATODA: RHABDITATA AND ASCARIDATA

The characteristics of the sub-class Phasmodia have already been defined.

### 27.I Sub-order 3. Rhabditata

From the point of view of the biologist, this sub-order is of outstanding interest, for it contains both parasitic and non-parasitic species and also many which are on the borderline between a free-living and a parasitic existence. Many parasitic species have free-living stages in their life cycle, a fact which some workers consider represents a phylogenetic recapitulation. Three genera have been especially well studied, *Rhabditis*, *Strongyloides* and *Rhabdias*, and although these contain forms which are not all strictly parasitic, they are worth studying both on account of the ease with which they can be obtained for laboratory study and for the light which they throw on stages in the life cycles of other important parasitic forms. *Rhabditis* may readily be maintained through all its stages *in vitro* and offers superb experimental material, especially in the field of nutritional studies.

The members of the sub-order are characterised by possessing a 'rhabditoid' pharynx with an anterior swelling followed by a posterior bulb (Fig. 117).

### 27.II Genus *Rhabditis*

The morphology of a typical example of this genus, *R. maupasi*, has already been studied (p. 286). Earthworms all over the world are parasitised by rhabditid larvae of this and related species in the 'dauer larva' stage and form a readily obtainable source of material. Invertebrates other than earthworms are also utilised by juvenile *Rhabditis*. Coprophagous insects are a particularly favoured host. Thus, *R. coarctata* is parasitic in or on dung beetles, and makes use of them as carriers to fresh dung in which final sexual development is achieved. *R. dubia* similarly makes use of dung-feeding psychodid flies.



*R. strongyloides* may establish itself in cutaneous ulcers in dogs. Several species of *Rhabditis* have been reported from man, but it is unlikely that these cases are more than accidentally temporary parasites, for in general they are coprozoic organisms.

### 27.12 Genus *Strongyloides*

Many vertebrates, particularly mammals, but also reptiles, birds and amphibians, are parasitised by members of this genus. *S. stercoralis* infects man, mainly in warm moist areas throughout the world. The most readily available source of laboratory material

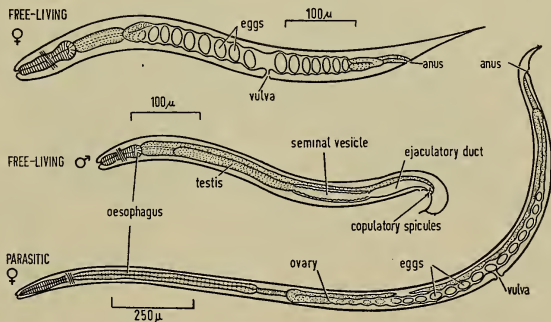


FIG. 134. *Strongyloides stercoralis*—morphology of parasitic and free-living generations (after Faust, 1949).

is the faeces of farm animals which contain eggs and larvae. The commonest species are *S. papillosus* in ruminants, *S. westeri* in equines, *S. ransomi* in pigs, and *S. ratti* in rats.

**Morphology and life cycle.** All species are intestinal parasites. They are slender and small and characterised by the possession of an unusually long oesophagus which in the female extends  $1/3$ – $2/5$  of the body length (Fig. 134). The anal opening is ventral and the tail is pointed. In the parasitic generation, the males are only known for a few species and it is likely that the females are usually, if not always, parthenogenetic. The parasitic male, where known, is about half the size of the female.

The female genitalia are double and the thin-shelled transparent eggs are either passed with the host faeces or hatch within the intestinal epithelium. In the latter case, the larvae are found in the faeces, often growing to twice or three times their hatched size by the time they reach the outside. Larvae are rhabditoid but without the typical medium bulbar swelling found in the true rhabditiform type (e.g. *Rhabditis*).



*Larval development* (Fig. 135). The fate of larvae passed in the faeces is determined to a large extent by the nutriment available in the environment. Indirect (heterogonic) or direct (homogonic) development can take place. In indirect development, larvae rapidly show sexual differentiation, and within thirty hours develop into free-living sexually mature male and female worms which resemble *Rhabditis* in their general morphology (Fig. 134). Rhabditoid larvae develop from the released eggs. After three to four days larvae moult and develop into the elongate infective filariform larvae. These infective larvae are clearly distinguishable from other rhabditoid stages by the very long cylindrical pharynx.

Under environmental conditions not defined, the rhabditoid larvae shed in the faeces may undergo 'direct' development into infective filariform larvae without undergoing sexual maturity. Both types of infective larvae become established in the host either by penetrating the skin or by oral ingestion. Those which penetrate the skin undergo a migration from the dermal tissues to the venous circulation and from there through the heart to the lungs—up the bronchi and trachea where they are swallowed and pass down into the intestine.

The females on reaching the duodenal mucosa burrow deep into the intestinal villi and even into the epithelium of Lieberkühn's glands. In the mucosa, they grow and produce eggs. In *Strongyloides stercoralis*, the parasitic males are apparently unable to penetrate the mucosa so that copulation, if it occurs at all, must occur in the lumen. In this species, also, the rhabditoid larvae of the parasitic female may become transformed into the infective filariform type and penetrate the intestinal mucosa or perianal region without external development being required (Fig. 135). This is an example of 'auto-infection'.

*Factors controlling heterogonic or homogonic development.* The factors which determine whether the life cycle of *Strongyloides stercoralis*, and related species, shall be heterogonic or homogonic are not properly understood. It was formerly thought that purely homogonic and heterogonic strains might exist but this has been disproved, as apparently heterogonic strains can give rise to homogonic strains and vice versa. The most recent work suggests that although the type of life cycle is determined by genetic factors in the eggs of parasitic females, external environmental factors play a considerable role in determining the final pattern of development. Factors such as temperature, humidity, osmotic pressure and nutritional properties of the medium being especially important. In *Strongyloides*, at least, every species is capable of undergoing homogonic or heterogonic development; under 'unfavourable' conditions, the tendency is to develop into infective filariform larvae, whereas under 'favourable' conditions, exclusively free-living forms are produced.

### 27.13 Genus *Rhabdias*

The best known member of this genus is *R. bufonis*, a common parasite in the lungs of frogs, toads and other amphibians (Fig. 136). It is a protandrous hermaphrodite with

the structure of a female. During the early male phase of development, sperms are produced and stored in the receptaculum seminis. During the later phase of maturation,

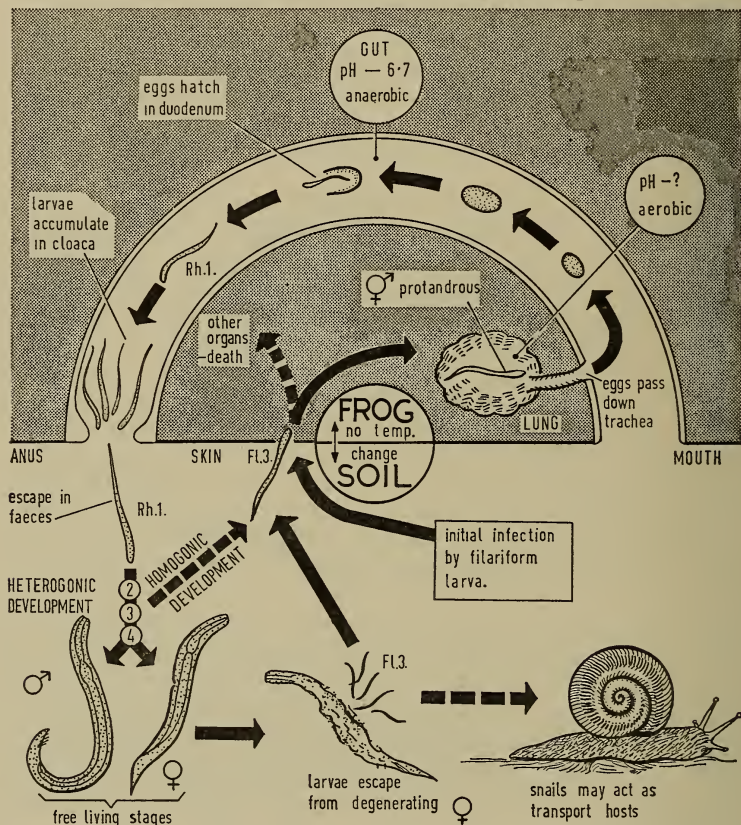


FIG. 136. *Rhabdias bufonis*—life cycle in frog; the numbers in the circles refer to the number of larval moults (original).

eggs are released, and pass from the lungs via the buccal cavity into the alimentary canal. Here they hatch into rhabditoid larvae with a typical rhabditoid pharynx. These larvae



are passed with the faeces and develop in the soil into males and females. After insemination, the females produce viviparously filariform larvae which devour the mother worm and escape as infective forms. These penetrate the skin of the amphibians and reach sexual maturity in the lungs.

#### 27.14 Genus *Neoaplectana*

This genus contains forms parasitic on insects. Like many other rhabditoids, it is a facultative parasite which may repeatedly complete its life cycle in decaying insect bodies, but when the food supply has been exhausted it will produce an infective filariform larva which penetrates into living beetles, both larvae and adults. Within the beetle gut, larvae become sexually mature and reproduce ovoviviparously. An infected beetle generally dies and the young nematodes feed on its carcass. The best known species is *Neoaplectana glaseri* which has been cultured through all its life cycle *in vitro* (p. 428).

### 27.2 Sub-order 4. Ascaridata

#### 27.21 Superfamily 1. Ascaridoidea

The members of this sub-order to which the term 'ascarids' is loosely applied, are relatively large nematodes which are obligatory intestinal parasites of vertebrates. They are mostly large, stout polymyarian worms. The eggs differ from those nematodes already described, being thick-shelled with an uneven surface (Fig. 127). Unlike the Rhabditata, the eggs rarely hatch externally but require to embryonate in an aerobic environment before development to an infective stage can occur. Hatching takes place only on ingestion by another animal.

It was formerly thought that the life cycle was direct but it has now been shown that several species utilise intermediate hosts in which larvae become encysted. For example, *Ascaris columnaris* in the skunk and *A. devosi* in the martin have rodents as intermediate hosts. In the case of direct infection, the larvae of some species undergo a remarkable migratory phase before returning to the intestine. In the case of *Toxocara canis* in the dog prenatal infection of the pups may result (Sprent, 1954).

In some cases, these migrations may damage the brain. Larvae causing such damage serve to increase the probability that the intermediate host will be caught by the final host. The rodent-damaging larvae thus appear to be of survival value to ascarid populations (Tiner, 1953).

*Genus Ascaris. Type Example: Ascaris lumbricoides*

The best known species of this genus is the so-called 'large roundworm', *Ascaris lumbricoides*, which is a parasite of man, some apes and pigs all over the world. The

species found in man and pig have long been considered to be morphologically identical, but minute differences have been recorded (see digestive system). They appear to be serologically identical, but they represent 'physiological strains' since the pig *Ascaris* will not develop in man or vice versa.

This conclusion was reached as a result of the daring experiments carried out by the two Koino brothers in 1922. One swallowed 2,000 embryonated ova of *Ascaris* from man, and the other 400-500 embryonated ova of *Ascaris* from swine. In the former case, over 600 worms were recovered on medication, but none in the latter case.

*External features.* Females (20-35 cm) are much larger than the males (15-31 cm) and the latter are also distinguished by a curved posterior end bearing a slit-like anal opening from which often protrudes a pair of copulatory spicules. The cuticle, which bears transverse markings, is sometimes brown in colour due to the presence of quinone-tanned proteins. The position of the excretory canals is marked by two broad brownish lateral lines, and the position of the main nerve cords similarly marked by dorsal and ventral lines just visible as white lines through the cuticle. Some 2 mm from the anterior end is an excretory pore, ventral in position and visible only in exceptional specimens. In the female, a narrow constricted band may be seen about one-third the distance from the anterior end, and this bears the slit-like opening of the vulva. In the male, there is a sub-terminal cloaca.

*Digestive system.* The mouth is surrounded by three cuticulate lips which are best seen in *en face* sections (Fig. 120). The dorsal lip bears a pair of papillae which represent the two papillae of the external circle (p. 290). The paired subventral lips bear (a) amphids, and (b) fused ventro- and latero-ventral papillae. The inner edges of the lips are provided with denticulous ridges whose structure is believed to differ in the human and pig varieties. The alimentary canal is the usual nematode type (p. 291) with the intestine proper bearing a well-marked bacillary layer (Fig. 122).

Sprent (1952) has shown that a morphological distinction exists between the denticulous ridges of the pig *Ascaris* and the human *Ascaris*. In the pig *Ascaris*, the denticles have straight edges and form a conspicuous row of more or less equilateral triangles. In contrast, the denticles of the human *Ascaris* are considerably less conspicuous, being smaller and having concave edges. These differences, however, are not always sharply defined.

*Muscular system.* This is built on the polymyarian plan with about 150 muscle cells in each quadrant. The vacuolated protoplasmic parts of the muscles are particularly well seen in transverse sections (Fig. 137).

*Pseudocoel.* This cavity contains a protein-rich fluid of characteristic unpleasant odour,



which sometimes induces allergic symptoms in students or technicians handling specimens.

**Reproductive system.** The female system is double, with thread-like ovaries passing into oviducts and finally into wider uteri opening at the vulva. The male system is single with a large seminal vesicle joining the rectum and spicule pouch just before the cloaca.

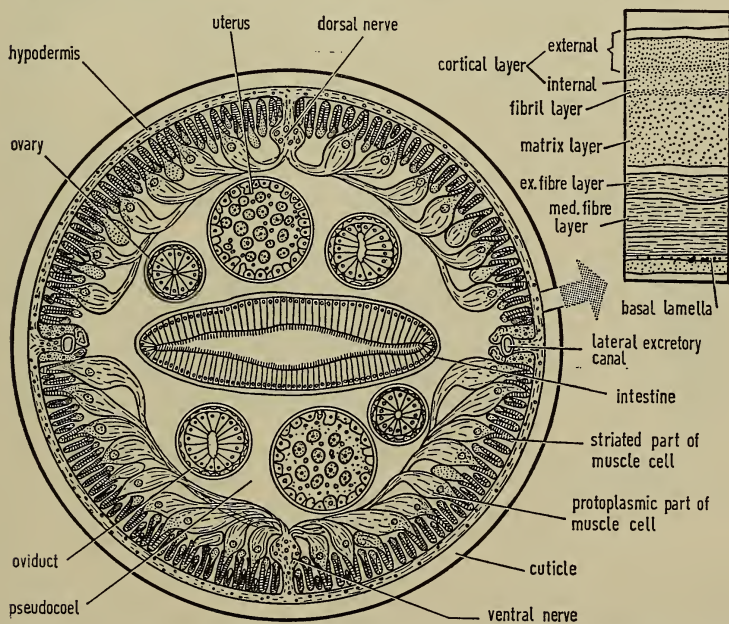


FIG. 137. *Ascaris lumbricoides*. Diagrammatic transverse section showing general morphology; the figure on the top right is an enlarged section of cuticle (after Brown, 1950).

The caudal end of the male bears some fifty or more pairs of simple pre-anal papillae and four pairs of single post-anal papillae, one pair of double post-anal papillae and one pair of phasmids.

**Excretory system.** This is often described as being of the  $\Pi$  type, but more recent studies suggest that it is of the H type, that is, with distinct anterior canals (Fig. 124). There is probably a nucleus in relation to the short terminal duct leading to the excretory pore

and another, the *giant sinus nucleus*, associated with the left lateral canal. Four coelomocytes (p. 302) occur in the body cavity situated anterior to the vulva, as shown in Fig. 138. *Life cycle* (Fig. 139). When eggs are passed in faeces, their further development is largely dependent on the oxygen tension, moisture content and temperature of their environment. In a cold dry atmosphere, it is said that eggs may remain viable for up to six years and in infected areas they may be widely distributed. At a temperature of 22–33°C.,

the eggs give rise to coiled rhabditoid larvae within 9–13 days, but do not hatch (except accidentally) until taken into the definitive host. The shell layers of the egg provide a remarkably resistant structure which can withstand many chemical agents, and in the laboratory, eggs are normally embryonated experimentally in 2 per cent formol solution. The motile rhabditoid larva undergoes a moult to produce the infective second-stage larva while still within the egg.

Infection is brought about by ingestion of the viable eggs containing the second-stage larva. Although man or pig are the natural hosts, eggs will hatch (see p. 12) in laboratory hosts such as rats or mice. On release within the intestine, the larvae undergo a migration so remarkable in character that it is difficult to believe that it is not a 'phylogenetic reminiscence', the parasite reliving its life cycle in a once intermediate, but now definitive, host. On hatching, the larvae burrow into the intestinal mucosa, penetrate a blood vessel and are carried within 24 hours to the liver via the hepatic portal vein. Here, they may remain for a few days, but then continue into the heart and thence

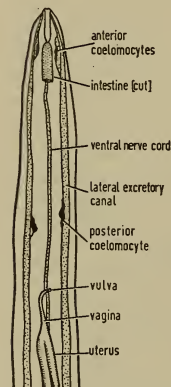


FIG. 138. *Ascaris lumbricoides*—anterior end dissected (after Chitwood, 1950).

via the pulmonary arteries to the lungs, being found there as early as 8 hours after infection. Within the lungs, they moult twice, break out of the capillaries into the alveoli, and finally work their way up the trachea to the pharynx and hence reach the small intestine. Here they undergo their final moult and become mature, some 8–9 weeks after the initial ingestion of eggs. Although the adult worms will not develop in rats, mice or guinea pigs, the migratory phases readily take place and can be easily followed by feeding infective eggs to these animals.

### Other *Ascarids*

Other common ascarids have a structure in general similar to *A. lumbricoides*, but the pattern of the migratory phase varies in the different species (Sprent, 1954).

In the genus *Toxocara*, the structure, host range and life cycle of the cat species has been worked out in detail by Sprent (1956).

He has shown that the eggs of this species can hatch and develop to the second-stage larvae within earthworms, cockroaches, chickens, mice, dogs, lambs and cats. Cats

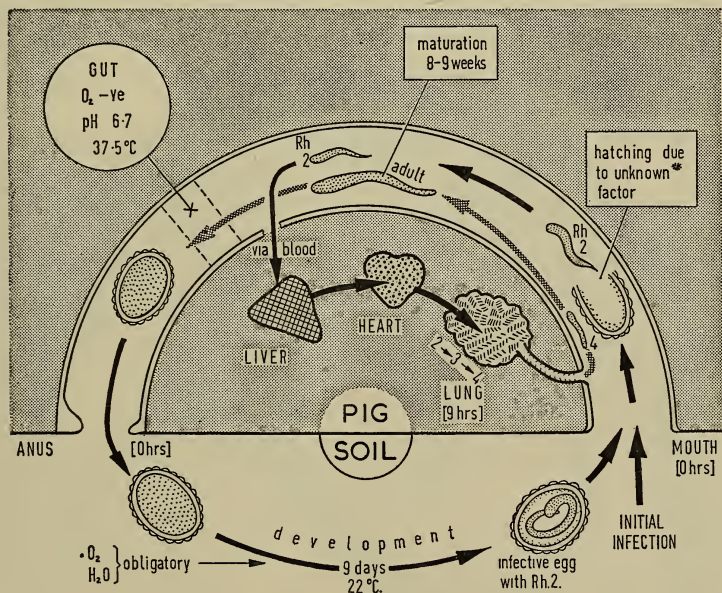


FIG. 139. *Ascaris lumbricoides*—life cycle in pig and some of the physiological factors relating to it. Hatching\* has been shown (Rogers, 1959) to be due to enzymes released under stimulus of the  $p\text{CO}_2$  in the host gut (original).

are probably normally infected in two ways, (a) directly, by ingesting embryonated eggs, (b) by eating mice harbouring larvae in the tissues. In the former case a migration similar to that of *A. lumbricoides* takes place, but in the latter case the larvae are almost entirely confined to the digestive tract. Prenatal infection was never found in *T. cati* although this is a common form of infection in the case of *T. canis*.

In the Anisakidae the pharynx or intestine or both may be provided with one or

more caeca. This group inhabits aquatic or fish-eating vertebrates. The life cycle is best known for *Contracaecum* in which fish, medusae, *Sagitta*, copepods, amphipods or cephalopods may act as intermediate hosts.

## 27.22 Superfamily 2. Oxyuroidea

The oxyuroid nematodes (Table 39) are chiefly characterised by possessing an 'oxyuroid' (=bulboid) type of pharynx, that is one provided with an end bulb (Fig. 121).

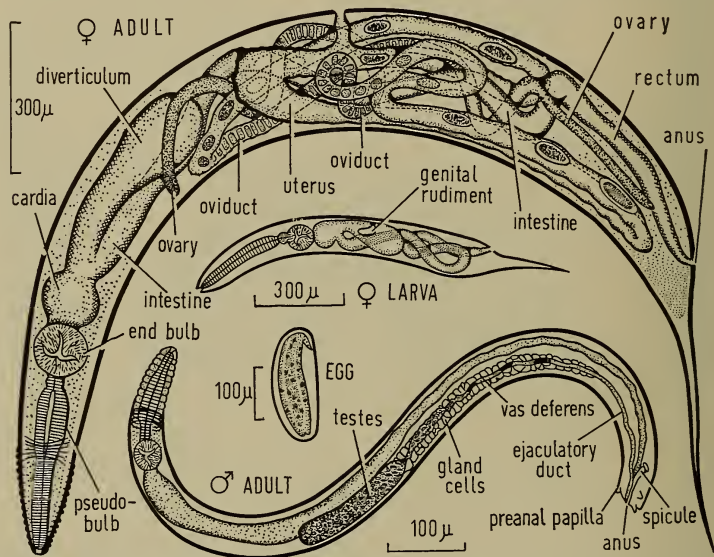


FIG. 140. *Leidyneria appendiculata*—an oxyuroid of the cockroach (after Dobrovolsky and Ackert, 1934).

They are small, rather transparent, meromyarian worms, with pointed tails. The species parasitising vertebrates inhabit the colon, caecum, or posterior region of the intestine, all regions of poor nutriment. The invertebrate species inhabit the intestine or malpighian tubules of insects.

*Type Example:* *Leidyneria appendiculata*

definitive host: *Periplaneta americana*

location: mid-gut



*Morphology.* The general morphology of the female which has been described by Dobrovolsky and Ackert (1934), is shown in Fig. 140. Points of especial interest are the closely annulated cuticle and the presence of a large intestinal diverticulum. The male

TABLE 39

## SOME COMMON OXYUROID NEMATODES

Species	Host
<i>Enterobius vermicularis</i>	man
<i>Syphacia obvelata</i>	rat and mouse
<i>Aspicularis tetraptera</i>	mouse
<i>Hammerschmidtiella diesingi</i>	<i>Periplaneta americana</i>
<i>Leidynema appendiculata</i>	<i>Periplaneta americana</i>
<i>Thelastoma bulhosi</i>	<i>Periplaneta americana</i>
<i>Thelastoma icemi</i>	<i>Periplaneta americana</i>
<i>Blatticola blattae</i>	<i>Blattella germanica</i>

likewise has an annulated cuticle but no intestinal diverticulum. The tail is characteristically curved. The testis is a compact structure near the middle of the body. There is a single spicule. Both have a cylindrical oesophageal pseudobulb.

*Life cycle.* The life history is direct as in other oxyuroids. The eggs when laid are unsegmented, but develop rapidly under the usual requirements of oxygen and moisture. Embryonation may be greatly accelerated by raising the temperature. At 37° C., the two-cell stage is reached within an hour and by twenty-four hours the active embryonated stage is reached. Infection is by direct ingestion of infective eggs which hatch in the post-intestine. Chitwood (1932) has given a synopsis of nematodes parasitic in insects of the family Blattidae.

#### Other species.

The second commonest species of oxyuroid in the cockroach is *Hammerschmidtiella diesingi* which may be distinguished from *L. appendiculata* by possessing an oval instead of a cylindrical pseudobulb. Its life cycle is similar to *Leidynema*.

*Syphacia obvelata*—an oxyuroid of the rat (Fig. 141).

This species occurs in the caecum of about 10–15 per cent of laboratory rats, but is less common in mice. Particular distinguishing features are: three broad lips placed

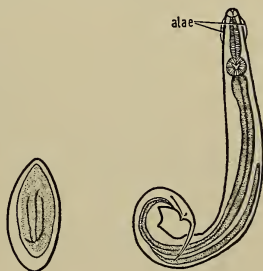


FIG. 141. *Syphacia obvelata*—male. An oxyuroid of rats and mice (adapted from Yorke and Maplestone, 1926).

in a tri-radiate position around the mouth; characteristic mamillons on the ventral surface; inconspicuous cervical alae; long attenuated caudal extremity; tail of male very curved, with conspicuous post-anal processes. The worms are ovoviviparous but, unlike the eggs of the Ascaridata, do not require a high oxygen tension for further development, so that embryonation to the rhabditoid stage normally occurs within the body of the female. Infection takes place by direct ingestion.

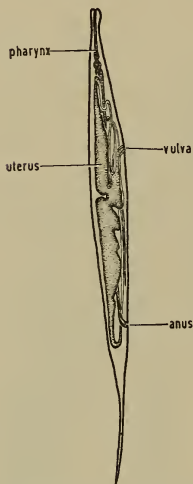


FIG. 142. *Enterobius vermicularis*—female. The 'pinworm' of man (after Faust, 1939).

*Enterobius vermicularis*—an oxyuroid of man (Fig. 142).

This is the human 'pinworm' or 'seatworm' widespread in temperate climates but, unlike most helminths, rare in the tropics. Probably every child in the temperate-zone areas has been infected not once, but many times in early childhood. Once it reaches a household, it is likely to infect every member of a family. Fortunately it is relatively harmless, although it may cause restlessness and irritability in young children. *Morphology.* The worms, which have an opalescent white appearance, are small and active, and have a typical oxyuroid morphology. The females measure 8–13 mm and the males 2–5 mm. The adults live in the caecum.

*Life cycle.* By some mechanism, not understood, the females are capable of restricting their egg laying to certain periods. At night, possibly stimulated by the extra warmth of bed-clothes, egg-laden females migrate down to the anus where, under contact with air, eggs are laid and the females retreat to the caecum once more. The newly laid eggs are sticky and adhere to the skin. Some worms fail to return to the anus, become dried on the skin and 'explode' to release quantities of eggs.

One of the features of infections with this worm is that eggs are rarely found in the faeces but may be found in skin scrapings near the anus region. The standard medical practice is to press a piece of Scotch tape on to the skin and stick this to a microscope slide. The eggs are like those of *Syphacia*, slightly flattened on one side and containing a rhabditoid larva. In a small number of cases, eggs may hatch in the anal region and migrate up the caecum to reach maturity. Such a mode of infection is termed 'retrofection'. In children, the intense itching caused by the migrating worms at night may cause them to scratch the anal region, so that the eggs may become lodged



under the finger nails and so reach the mouth. This is, however, probably the least important means of infection. The eggs are exceptionally light and easily airborne, and rapidly become distributed throughout a normal household or institute, often in spite of the most elaborate hygienic precautions. In one household examined, up to 90 per cent of samples of dust taken from different parts contained eggs of *Enterobius* (Nolan and Reardon, 1939). Enterobiasis (=oxyuriasis) has been the subject of an exhaustive investigation by the U.S. National Institute of Health (1937-43).

#### *Other Oxyuroids.*

In the genus *Ascaridia*, the males have a rimmed pre-anal sucker. The best-known species is *Ascaridia galli*, a common parasite of chicks. This species does not undergo a tissue migration, but merely temporarily buries itself in the mucous membrane and later becomes free.

In the genus *Heterakis*, both males and females bear cervical alae and the male bears caudal alae and twelve pairs of papillae. *H. gallinae* of chickens is of considerable economic importance and biological interest, as its eggs act as a carrier of the protozoan *Histomonas meleagridis* (p. 47), although the precise location of the parasite in the egg has never been observed. This genus and the previous one are placed by some authorities in the Ascaroidea.

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## CHAPTER XXVIII

### PHASMID NEMATODA: STRONGYLATA

The most striking diagnostic feature of this group is the possession by the male of a copulatory 'bursa' a posterior umbrella-like cuticular expansion which is absent from the males of all the other sub-orders of nematodes (but see p. 289). Many of the strongyloid nematodes are of considerable economic importance, attacking mammals, including man and domestic animals. The eggs hatch outside the body and the free-living larvae, after the usual moults, reinfect via the skin or mouth, or more rarely, by means of an intermediate host.

#### 28.1 Type Example: *Nippostrongylus muris*—the rat hookworm

This nematode, originally known as *Heligmosomum muris* (Yokogawa, 1920, 1922), occurs naturally as an intestinal parasite of the wild rat *Epimys rattus norvegicus*, but it may be transmitted experimentally to laboratory rats, cotton rats, rabbits and hamsters, although stunted or abnormal development may occur in some of these hosts. On account of the ease with which it may be maintained through all its stages in the laboratory, this organism is especially suitable for experimental work. The adults occur in the anterior region of the intestine, either in contact with the mucosa or partly embedded in it.

*General morphology.* The morphology of the worms is shown in Fig. 143. The males measure 3–4 mm, and the females 4–6 mm in the wild rat. In aberrant hosts, such as the cotton rat, growth is stunted and the reported size ranges are: males 1.7–3.0 mm, females 1.7–3.4. The head is small and bears a cephalic expansion of the cuticle. The cuticle has transverse striations and ten prominent longitudinal ridges. The mouth and buccal cavity are both small; the subcuticular part of the circumoral area has the form of two small teeth, one on each side. The armed buccal cavity enables the nematode to take a firm hold on the mucosa from which it draws blood. Recently fed worms thus appear blood-red in colour. The excretory system is well developed and hides the anterior region of the gonads. It consists of two elongate sacs, which open externally

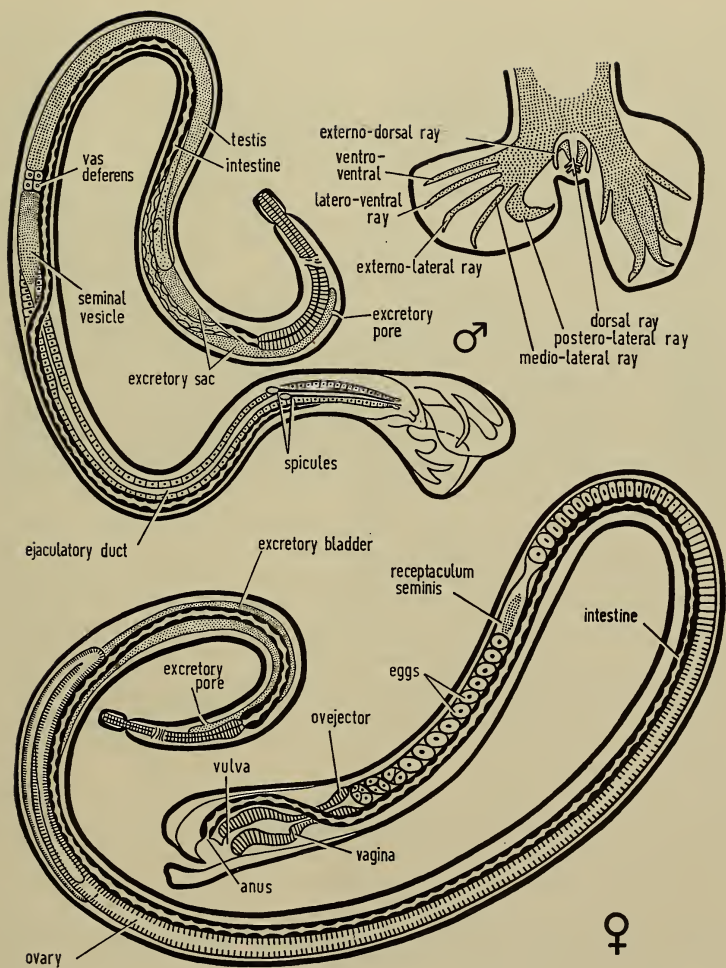


FIG. 143. *Nippostrongylus muris*—anatomy of male and female (adapted from Yokagawa, 1920).

by an excretory pore just in front of the base of the pharynx. The nerve ring lies just posterior to the excretory pore. The intestinal cells contain a melanin-like pigment.

*Male.* The characteristic external feature is the bursa, which forms an umbrella-like expansion surrounding the cloaca. It consists of two large lateral lobes and a small dorsal lobe. The lateral lobes, which are each supported by fleshy rays comparable to the ribs of an umbrella, are asymmetrical, the right being larger than the left. The left-lobe rays are more divergent than those of the right. The terminology used in naming the rays is shown in Fig. 145. The dorsal ray, supporting the small dorsal lobe, terminates in four digitations.

The testis occupies much of the anterior half of the body; its beginning is difficult to see, being hidden by the large excretory sac. It passes into the usual male organs, vas deferens, seminal vesicle and ejaculatory duct; the distal end of the latter contains two yellow-brown spicules. The distal end of the intestine joins the posterior end of the ejaculatory duct.

*Female.* The female is larger than the male. The vulva is posterior in position and opens on the ventral surface just in front of the anus. When contracted, the posterior body region is withdrawn to such an extent that the cuticle forms a sac surrounding the vulva and anus. The anterior end of the single ovary is bent and looped. The main part of the ovary is dorsal and is filled with a single row of developing oocytes gradually increasing in maturity as they approach the receptaculum seminis. The uterus, which occupies much of the posterior part of the body, is connected posteriorly with a muscular ovejector leading into the vagina. The latter is lined with cuticle. The eggs are ellipsoidal with a very thin shell, and an average size range of  $58\mu \times 33\mu$ . In the uterus, they develop to the 1-16 cell stage, and when passed in the faeces they may be at the 4-16 cell stage, or occasionally the morula stage. Lucker (1934) has given an account of the preparasitic moults and Schwartz and Alicata (1934) have described the development in the rat.

*Life cycle.* The life cycle involves an external non-parasitic phase in an aerobic, humid environment at air temperatures followed by a parasitic phase in the anaerobic, warm-blooded environment provided by the intestine. Normal development of the eggs and larvae in the soil requires abundant oxygen and moisture. These conditions may be provided in laboratory experiments by mixing eggs with charcoal or alumina and spreading on moist filter paper (Fig. 144). Hatching of the rhabditiform larva takes place at room temperatures (18-22° C.) in about 18-24 hours. The first rhabditiform larva grows and moults to the second rhabditiform larva within about 48 hours. This

in turn grows, moults within 4–5 days and gives rise to the third-stage filariform larva. This larva normally exsheaths while still on the surface of the charcoal culture, unlike

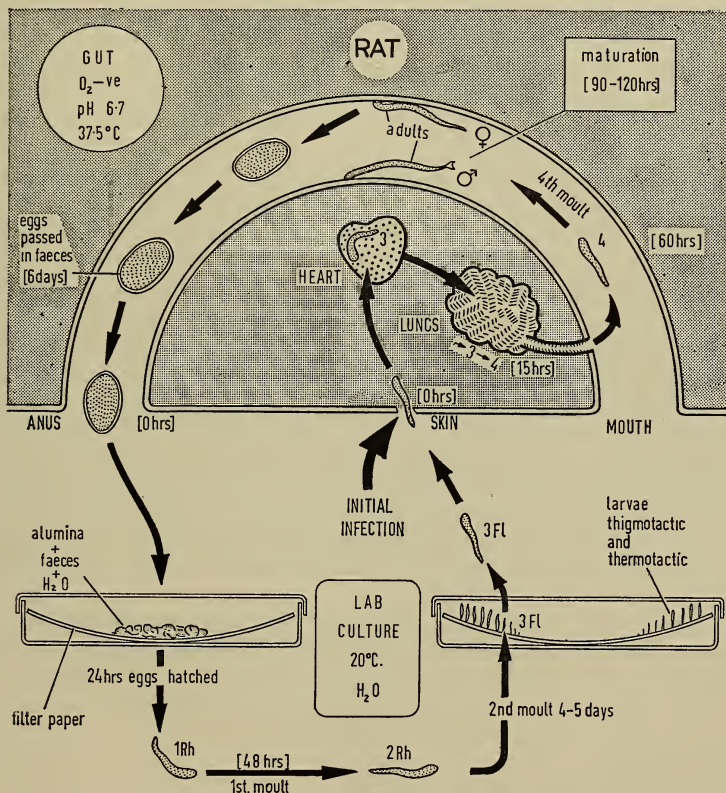


FIG. 144. *Nippostrongylus muris*—life cycle as carried out in the laboratory (original).

many other strongyle larvae, which exsheath only when taken into the host gut. The cycle from egg to infective filariform larva normally requires about 4–5 days, but the time naturally varies with temperature.



The filariform larvae show tropisms which are characteristic of all strongyle larvae. They are markedly thermotactic and are rapidly stimulated into activity by the warmth of a nearby animal. They also show remarkable negative geotropism. This is clearly shown in laboratory faecal cultures. In such cultures, larvae migrate to the edge of the moist filter paper, where on reaching the highest points, they extend themselves into the air and wave back and forth slowly. In the natural state, they likewise climb to the top of soil particles and await a suitable host. Both these tropisms would tend to increase the chance of infecting a passing host.

Infection of the rat host is accomplished by placing larvae directly on the skin and allowing them to penetrate, or by hypodermic injection, or via the mouth, the latter method being rather ineffective. About 5,000 larvae on the skin give a heavy but not fatal infection. Hypodermically, a much smaller dose is effective. After entry into the blood stream, larvae are carried via the heart to the lungs within about 11–20 hours (usually about 15). Here the larvae feed on whole blood and undergo rapid growth and differentiation culminating in the third moult to the fourth-stage larva. The latter are carried by ciliary action up the bronchi and trachea, finally passing down the pharynx to the intestine. The first larvae reach the intestine in about 41 hours and about 50 per cent arrive there between 45 and 50 hours (Twohy, 1956). Here the fourth and final moult takes place resulting in fifth-stage male and female worms. Maturation is rapid and by the sixth day following infection, eggs appear in the faeces. Within the host gut, worms feed mainly on blood and tissue cells, but intestinal contents must also form part of the diet, since intestinal flagellates have been found within the gut lumen of the worms. About 60 per cent of the larvae which penetrate the skin survive passage through the tissues and reach the gut.

*N. muris* may be cultured through its entire life cycle *in vitro*, although normal development of the adult parasitic stages has not yet been obtained (see p. 429).

## 28.2 Sub-order 5 : Strongylata

### *Important families:*

Family Trichostrongylidae (trichostrongyles)

(e.g. *Nippostrongylus muris*, *Haemonchus contortus*).

Family Strongylidae (e.g. *Strongylus equinus*).

Family Ancylostomidae (hookworms) (e.g. *Ancylostoma duodenale*).

Family Metastrongylidae (metastrongylids = 'lungworms') (e.g. *Metastrongylus* sp.)

Family Syngamidae (gapeworms) (e.g. *Syngamus trachea*).



### 28.21 Family Trichostrongylidae

The trichostrongyles are small slender intestinal worms without leaf crowns (see Strongylidae, below) or cutting plates and with buccal capsules poorly developed or lacking. The family includes the rat hookworm, *Nippostrongylus muris* already described (p. 326), the stomach worm *Haemonchus contortus*, and a number of other species of veterinary importance.

*Haemonchus contortus*. This species is a blood-sucking nematode which occurs in the fourth stomach (abomasum) of the sheep and other ruminants, a highly acid environment with a pH of approximately 3.0. In general structure, it resembles *Nippostrongylus muris* and is often reddish or striped in colour due to contained blood. Females are 18–30 mm long; males, 10–20 mm.

*Life cycle*. Eggs are passed in the faeces and hatch in favourable climatic conditions. Like *Nippostrongylus*, three non-parasitic larval phases occur, but the third larva, after the second moult, retains its skin so that it is unable to feed, but survives at a low metabolic rate on its food reserves. The infective larva is unable to penetrate the skin and must be taken directly into the mouth; grazing animals ingest them accidentally with their food. On reaching the abomasum, it undergoes the third and fourth moults and reaches maturity. Many thousands of worms may occur in a single ruminant stomach, and it has been estimated that 4,000 worms suck about 60 ml of blood per day. Medium infections cause sheep and cattle to lose condition and heavy infections may cause death. Development of immunity to *H. contortus* was first demonstrated by Stoll in 1928, and his original experiment, resulting in 'self-cure', is considered further on p. 384.

*Other Trichostrongyles*. Farm animals are infected with a number of other Trichostrongylidae with essentially the same life cycle as *H. contortus*. They differ in their host preferences and the part of the alimentary canal parasitised. Their distribution, in a world-wide sense, is largely determined by the conditions under which the free-living larvae can develop and survive. Sheep or cattle droppings (conveniently obtained from an abattoir) frequently contain eggs and larvae and serve as useful demonstration and experimental material; the latter may be separated by using a Baermann funnel.

### 28.22 Family Strongylidae

The typical features of this family are the well-developed buccal capsule, with a thickened ridge on its dorsal side, the *dorsal gutter* which represents the opening of the dorsal oesophageal gland, and one or two rows of circum-oval extensions of the cuticle termed *leaf-crowns* or *corona radiata*. As in the Ancylostomidae, teeth are present. Many members of this family are pathogenic to domestic animals, and all have a life

cycle in general similar to *Haemonchus contortus*, although the path followed by migrating larvae in some cases is still a matter of dispute.

One of the most interesting genera is *Strongylus*, which includes strongyles of horses, often termed 'red worms'. The early larval stages of some species are undergone in 'nodules' in the alimentary canal, from which the fourth-stage larvae escape to undergo complicated migrations; in some species, development occurs in the peritoneal cavity. Another group of 'nodular worms' are the Oesophagostominae (oesophagostomes) which have a shallow buccal cavity and a cervical groove. They are parasites of pigs, sheep and cattle as well as monkeys and apes, and occasionally man.

### 28.23 Family Ancylostomidae

The hookworms which make up this family are chiefly distinguished from other strongyles by the possession of two ventro-lateral cutting plates often bearing teeth. The life cycle in general resembles that of *Nippostrongylus muris* (p. 328). There are a number of genera, the best known being *Ancylostoma* and *Necator*.

#### *Hookworms of man*

Hookworms must be classified as one of the most destructive of human helminth parasites. They insidiously undermine the health of their hosts, causing stunting of growth and general laziness often accompanied by acute mental distress. They occur in predictable areas where sanitary and environmental conditions favour the development of eggs and the infection of hosts. This is roughly throughout the tropical and subtropical world. There are two main species attacking man:

*Ancylostoma duodenale*: essentially a northern species, occurring in the northern districts of China and Japan, western Asia and in Europe and North Africa.

*Necator americana*: the so-called 'American' hookworm, essentially the predominant species in all parts of the tropics, except those mentioned above.

The original distribution of both these worms has been changed considerably by migrations of peoples.

*Morphology*. The general morphology and details of the life cycle resemble those of *Nippostrongylus* but the worms are roughly twice the size (males 8–11 mm; females 10–13) and the vulva opening is almost one-third of the body length from the posterior end; the female has two ovaries. The conspicuous buccal cavity is armed with a pair of chitinous plates which either bear teeth (*Ancylostoma*) or have a sharp blade-like edge (*Necator*). The head is curved in both species, but in necators is finer than in the ancylostomes and more sharply bent. The bursae in the males of both species are well devel-

oped, that of *Necator* being distinguished from *Ancylostoma* by the split dorsal ray and close arrangement of the lateral rays (Fig. 145).

*Life cycle.* The life cycle follows closely that of *Nippostrongylus*. The adults live in the small intestine, firmly attached to the mucous membrane, and feed on blood and tissue. Large numbers of eggs are produced (up to 20,000 per day in the ancylostomes) and are passed out with the host faeces. In favourable conditions of moisture, temperature

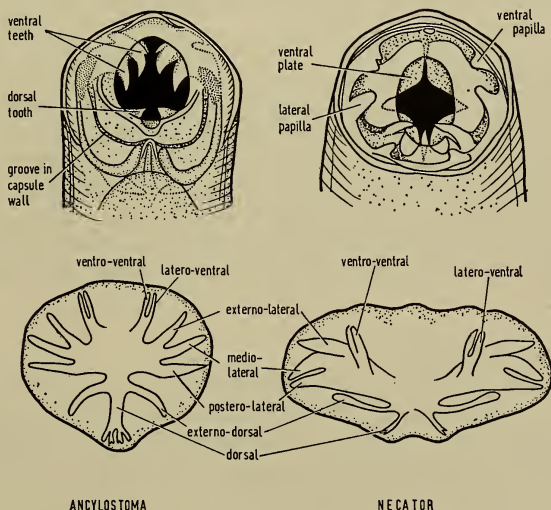


FIG. 145. Comparison of the buccal cavities and bursae of *Ancylostoma* and *Necator* (adapted from Brown, 1950, and Chandler, 1955).

and oxygen they then hatch as rhabditiform larvae which feed on bacteria. After two moults, these larvae become infective and climb to the highest part of the moist ground awaiting a barefooted victim. Like *Nippostrongylus*, moderate heat or touch stimulates them into activity and these responses must increase their chances of reaching a host. The food reserves of the third-stage infective larvae are small so that their free-living life is short. Human infection normally takes place through the skin and, less frequently, from infected water or food. On reaching the blood stream, a migration similar to that of *Nippostrongylus* occurs. Larvae swallowed may not show this migration.

As hookworms are essentially blood suckers, it is important to note that the degree of pathogenicity depends largely on the size of the infection. A few hookworms present in the mammalian gut do little harm to a well nourished host, but a worm burden of 500 or so in an undernourished host can produce profound effects. Thus nutritional considerations play a marked part in the evaluation of this organism as a pathogen. Its main biological effect is the depletion of blood, and this must be made good by the feeding of quantities of iron in a suitable form to the host, and also assuring adequate supplies of protein.

#### 28.24 Family Metastrongylidae

This family includes the so-called 'lung worms', slender worms which inhabit the respiratory tract of sheep, goats, cattle, horses and pigs. In contrast to the hookworms, the first larva, not the egg, is usually shed and after two moults becomes infective. Infection is by ingestion. The best-known genus is probably *Dictyocaulus* which has a simple direct life cycle. Other metastrongyles require slugs or snails as carrier hosts. *Metastrongylus apri* is of biological interest as being the vector of swine influenza. Its life cycle is indirect, and it can survive for long periods (up to three years) encapsulated in earthworms.

#### 28.25 Family Syngamidae

These are bright red worms which live in the trachea and bronchi of mammals and birds, sometimes with pathological effects. The male and female remain *in copula* so that the whole is Y-like in appearance. The best-known species is *Syngamus trachea*, a parasite of fowl, turkeys and other domestic birds (Taylor, 1935). It is also a common parasite of wild birds in European countries but not in the United States.

The eggs are coughed up, swallowed and passed out with the host faeces. The usual early larval stages occur while still within the egg, so that when hatching does occur, the infective third larva has developed. This may either be eaten directly by a bird host or by an invertebrate 'transport' host in which it encysts but undergoes no further development.

These transport hosts may be earthworms, slugs, snails, insects and possibly other arthropods. Transport hosts not only protect larvae from climatic conditions to which they may be susceptible but also serve to distribute them over a wider area than the rather inactive larvae could cover by their own efforts. When ingested by suitable bird hosts, the infective larvae reach the lungs, probably by way of the circulatory system, and develop to maturity.

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## CHAPTER XXIX

### PHASMID NEMATODA:

### SPIRURATA, CAMALLANATA

#### 29.1 Sub-order 6: Spirurata

Members of this sub-order of nematodes require intermediate hosts for the completion of their life cycle. The pharynx is cylindroid with an anterior muscular portion and a posterior glandular portion (Fig. 121); the males have well developed alae and spirally coiled tails. They are divisible into two superfamilies: the Filarioidea whose members have a small mouth, usually without lips or a buccal capsule and with the vulva far anterior; and the Spiruroidea, whose members usually have a cuticularised vestibule, two to four paired lips and the vulva in the middle or posterior region of the body.

#### 29.11 Superfamily Filarioidea

The *Filaria* represent a group which has successfully invaded the blood stream, connective tissue or serous cavities of vertebrates; they are long, thread-like forms. Many species are of medical or veterinary importance, attacking man and domestic animals to which they are transmitted by haematophagous arthropods, often mosquitoes. The sexually mature females release swarms of pre-larval stages, termed *microfilariae*, into the peripheral blood. Most species are ovoviviparous and some have 'sheathed' microfilaria; the sheath is probably the ruptured 'shell' still attached to the worm. Some filaria of birds are oviparous.

#### 29.12 Type Example: *Litomosoides carinii*

definitive host:	the cotton rat, <i>Sigmodon hispidus litoralis</i>
intermediate host:	a mite, <i>Bdellonyssus (Liponyssus) bacoti</i>
location:	pleural cavity



*Natural occurrence.* This filariid occurs as a natural parasite of various sub-species of the cotton rat *Sigmodon hispidus litoralis*, a creature of open grassland areas in the United States and S. America. The rats are parasitised by numerous ectoparasites (fleas, lice, ticks and mites) but only one species of mite, *Bdellonyssus (Liponyssus) bacoti*, has, so far, been shown to act as intermediate host.

*Site of infection.* In natural infections, the adult worms occur massed together in the pleural cavity, but occasionally invade the peritoneal space, especially in laboratory infections established by subcutaneous insertion.

*Morphology.* The worms are thin and long; females 50–130 mm; males 24–26 mm. The buccal cavity is narrow and the male tail lacks alae or papillae. In the female, there is a long ovejector, with a bulbous enlargement near the vulva.

*Life cycle* (Fig. 146). The mature females ovoviviparously discharge slender *microfilariae* into the pleural cavity. These are essentially pre-larval stages, which will not undergo further development until taken into the haemocoel of the intermediate host. From the pleural cavity, the microfilariae (which are sheathed) migrate to the blood stream by a variety of routes, the most common being via the lungs. They can, however, burrow between and into muscle fibres and may be found in small numbers in the cardiac muscle fibres of the ventricles.

The intermediate host is the tropical rat mite *Bdellonyssus (Liponyssus) bacoti*. When infected blood is taken up by the mite, the microfilariae burrow through the intestinal wall into the haemocoel. By about the eighth day in the mite, the first larva becomes typically sausage-shaped and undergoes its first moult. The second moult takes place on the ninth or tenth day and the larva grows further to a size of about 500–950  $\mu$  to become a fully infective third-stage larva about the 14th or 15th day. These times are based on development in mites maintained at 23–25° C., but variations can occur. The mode of infection is uncertain but it is probably through the skin rather than by ingestion of mites. Within the rat blood, an infective larva migrates to the pleural cavity and there continues its development for about one week before moulting to the fourth-stage larva. This larva undergoes considerable growth during the next 17 days, taking on the main characteristics of the male or female worm, with size ranges of approximately  $6.4 \pm 0.5$  and  $8.7 \pm 0.2$  mm respectively. At the end of this period (i.e. about 23–24 days after the initial infection of the rat) the final moult takes place and growth to the adult stages commences. The worms become sexually mature within 70–80 days and microfilariae may then be detected in the peripheral blood. The period required for maturation of *Litomosoides carinii* in the definitive host is less than for most other filariid worms, the majority of which require from 9–12 months to mature. The normal

peak of microfilarial production is between 17–20th week after infection and the adults may live for approximately 60 weeks or more. *L. carinii*, unlike some of the other

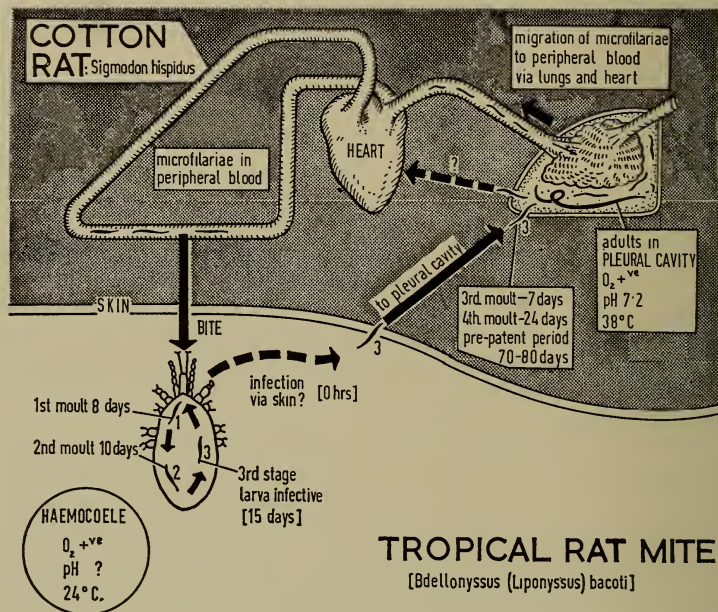


FIG. 146. Life cycle of *Litomosoides carinii*, a rodent filariid readily maintained in the laboratory. The times given for the various phases are minimal and considerable variations can occur (original).

filarial worms attacking mammals, does not exhibit microfilarial periodicity (see p. 343). The life cycle and biology of this worm has been the subject of a number of studies, chief of which are those of Chandler (1931); McDonald and Scott *et al.* (1951–53b); Williams (1948); and Bertram (1953).

### 29.13 Other Filariae

#### *Dirofilaria immitis*

This species occurs in the right ventricle and pulmonary arteries of dogs, cats, wolves, foxes and other carnivores in the tropics and sub-tropics. It has been reported in the British Isles.

*Life cycle*

Many species of fleas and mosquitoes can act as intermediate hosts. The general pattern of the life cycle resembles that of *Litomosoides*. The prepatent period is about 32 weeks and when microfilariae become patent they show a partial periodicity. They reach a maximum and a minimum concentration at certain times, 6 p.m. and 6 a.m. respectively. The minimum is never as low as in species showing complete periodicity (p. 342).

*Dipetalonema repens*. This species lives under the skin of dogs especially in the regions of the trunk, thighs and neck. It has a life cycle similar to *D. immitis* and, like it, may be transmitted by a range of arthropod vectors. It has a prepatent period of 25 weeks and a partial periodicity with a maximum peak about midnight, and minimum (about 20–40 per cent of the maximum) about midday.

**29.14 Filariae of Man**

There are a number of species of filariae parasitising man which occur in a wide range of tissue habitats: lymph glands, deep connective tissue, subcutaneous tissue or mesenteries (Table 40). Habitats of this kind particularly lend themselves to inflam-

TABLE 40  
SOME REPRESENTATIVE FILARIID NEMATODES

Species	Definitive host	Habitat	Intermediate host
<i>Litomosoides carinii</i>	rats	pleural cavity	<i>Bdellonyssus bacoti</i>
<i>Wuchereria bancrofti</i>	man	lymph glands	<i>Culex</i> spp., <i>Aedes</i> spp. <i>Anopheles</i> spp.
<i>Wuchereria malayi</i>	man	lymph glands	<i>Mansonia</i> spp.
<i>Loa loa</i>	man	subcutaneous tissue	<i>Chrysops</i> spp.
<i>Dipetalonema perstans</i>	man	deep connective tissue	<i>Culicoides</i> spp.
* <i>Dipetalonema streptocerca</i>	man	deep connective tissue	<i>Culicoides grahamii</i>
<i>Mansonella ozzardi</i>	man	mesenteries	<i>Culicoides furens</i>
<i>Onchocerca volvulus</i>	man	mesenteries	<i>Simulium</i> spp.
<i>Onchocerca reticulata</i>	horse	ligamentum nuchae	<i>Culicoides nubeculosus</i>
<i>Dirofilaria immitis</i>	dog and other carnivores	heart	<i>Anopheles</i> spp. <i>Culex</i> spp. <i>Myzomyia</i> spp. <i>Myzorrhynchus</i> spp. <i>Aedes aegypti</i> <i>Ctenocephalides</i> spp. <i>Pulex irritans</i> <i>Aedes aegypti</i>
<i>Dipetalonema repens</i>	dogs	subcutaneous tissue	

matory reactions, and this is a typical symptom in most human filarial infections. In some cases infections give rise to revolting fleshy deformities which are collectively known as *elephantiasis*. This is partly due to the inflammation of the walls of the lymphatics and the consequent hyperplasia, and partly due to mechanical blockage by the worms. Many species exhibit marked periodicity; this question is discussed further on p. 342.

### *Wuchereria bancrofti*

This organism occupies an important place in the history of parasitology, as Manson's discovery in 1878 that a mosquito intermediate host was required in its life cycle was the first proof of transmission of a human blood parasite by an arthropod.

Manson actually believed that larvae emerged from mosquitoes into the water during egg-laying and that man became infected by drinking infected water!

The distribution is limited to the tropical and sub-tropical countries chiefly in Asia, Africa and America, with a long intermediate-host season and high humidity. It also occurs in Australia and the Mediterranean area between 40° N. and 30° S.

*Habitat.* The sexually mature adults lie inextricably coiled in the lymph glands or ducts. *Life cycle.* Similar to that of the rat filariid *Litomosoides carinii* in most respects except that the arthropod hosts are various species of *Culex*, *Aedes* and *Anopheles*, and that the appearance of the microfilariae in the peripheral blood exhibits a marked nocturnal periodicity. Larvae reach their maximum concentration in the blood between 10 p.m. and 4 a.m., whereas during the daytime the embryos are concentrated in the lungs. Like the larvae of *Litomosoides*, the microfilariae are ensheathed in their egg membrane as are the majority of filariae of man showing periodicity (Fig. 149). On ingestion by the mosquito host, the sheath is lost and the microfilariae migrate rapidly to the thoracic muscles where further growth and differentiation takes place. In the muscles they become characteristically sausage-shaped. The infective stage is reached after the usual two moults, the process of maturation requiring some two weeks for completion. The infective larvae migrate into the proboscis and escape onto the skin and penetrate through the bite wound during the short period that a mosquito is biting. Growth is exceptionally slow and maturation to a sexually mature worm requires about nine months. In the Polynesian Islands and the Philippines there exists a non-periodic variety, the so-called *pacifica* variety.

*Wuchereria malayi.* This species occurs in Indo-China, Malaya, Indonesia, India and Ceylon. In general structure and life history it resembles *W. bancrofti* except for minor points in the anatomy of the male and the microfilariae. It is transmitted by mosquitoes of the genera *Mansonia* and *Anopheles*.

*Loa loa*

The 'eye' worm of West and Central Africa. It occurs mainly in the subcutaneous tissue of man and monkeys. The adults in man migrate in the subcutaneous connective tissues, a habit which from time to time leads them to cross the eyeball under the conjunctiva (hence the popular name). In monkeys they have also been found in the connective tissue surrounding the muscles.

*Morphology.* The general anatomy differs from *W. bancrofti* mainly in that the cuticle is covered with wart-like processes.

*Life cycle.* The intermediate hosts are certain species of *Chrysops*, the mangrove or 'softly softly' fly. Like *W. bancrofti*, this species exhibits periodicity but in this case it is diurnal, the larva almost disappearing from the blood at night. Associated with the infections are cutaneous swellings, 'Calabar swellings', probably developed as allergic reactions to metabolic by-products from the worms. These swellings may appear suddenly, last a few weeks and reappear in another site. A strain in monkeys has a different periodicity, probably associated with different *Chrysops* spp. as vectors.

*Dipetalonema* (= *Acanthocheilonema*) *perstans*. This is a non-pathogenic species in man and apes which occurs in Africa and S. America. It is located in the deep connective tissue and serous cavities and its unsheathed microfilariae do not exhibit periodicity. It is transmitted by midges of the genus *Culicoides*.

*D. streptocerca*. This species occurs in the Gold Coast, Cameroons and Congo. The adults are poorly known, and occur in the cutaneous tissue of man and chimpanzee. The microfilariae, like the previous species, do not exhibit periodicity. The vector is *Culicoides grahamii*, and probably other *Culicoides* spp.

*Mansonella ozzardi*

A non-pathogenic species occurring in the West Indies and northern parts of S. America. The adults, which are thin, thread-like worms often 2 feet long, occur in mesenteries and visceral fat; the male is almost unknown. Members of this genus are parasitic in man, horses, cattle and antelopes, the species in man and animals being morphologically indistinguishable. The microfilariae are unsheathed and occur only in the superficial layers of the skin which are penetrated by the midge vector, *Culicoides furens*. The parasites cause nodules in the skin of the vertebrate hosts.

*Onchocerca volvulus*

This species occurs in Central America and Central Africa.

*Habitat.* Mainly in subcutaneous tissues and subcutaneous nodules but also in lymph spaces.

*Life cycle.* The life history is similar to that of *W. bancrofti* except that the intermediate hosts are various species of blackflies of the genus *Simulium*. The microfilariae, which



are unsheathed (Fig. 149) and non-periodic, migrate to the lymphatics of the skin. Developing worms become enclosed by an inflammatory reaction of the host tissue, in a nodule of fibrous tissue in which they may become inextricably entangled. The larvae frequently damage the tissues of the eye (cornea, iris, conjunctiva and optic nerve) which may result in partial or total blindness. The African and American varieties vary somewhat in their preferences for parts of the body, the latter chiefly producing nodules on the head, while the former produces nodules in other regions, especially those of the hips and thighs. Hawking (1950) has reviewed recent work on filariasis.

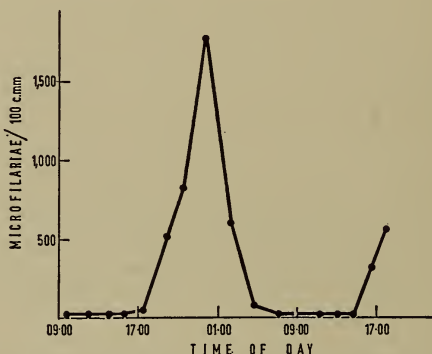


FIG. 147. Complete periodicity exhibited by microfilariae in a monkey; species unidentified but resembling *Dipetalonema sunci* (after Hawking and Thurston, 1951).

### 29.15 Identification of Microfilariae

Microfilariae as seen in blood films show certain diagnostic characters. One of the most striking of these is the presence or absence of a sheath—a delicate, closely-fitting membrane, probably the egg capsule, which is only detected when it projects beyond the head or tail of the larva (Fig. 149). Other diagnostic features depend on certain fixed points shown in Fig. 148.

### 29.16 Periodicity

As already indicated, the microfilariae of various species exhibit periodicity to a varying extent and this phenomenon has been investigated extensively. In the human strains of *W. bancrofti* and *Loa loa* and various other species (Fig. 147), it is well marked,



the microfilariae reaching a maximum concentration in the peripheral blood during the day and night respectively. In *Dirofilaria* (of dogs) it is less well marked, that is, there is a nocturnal periodicity, but not so marked as with *W. bancrofti*. In others, such as *Litomosoides carinii*, there is no periodicity at all.

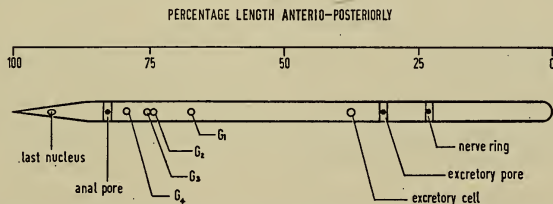


FIG. 148. Diagram of microfilaria, showing the chief points used in identification; G<sub>1</sub>-G<sub>4</sub> represent genital rudiments (based on Newton and Wright, 1956).

Two main problems arise in connection with the phenomenon of complete periodicity: firstly, is there a particular tissue site in the body where the microfilariae are located when not in the blood stream, and if so do they return to this site after migration? Secondly, what factors are responsible for the phenomenon of periodicity?

These are problems of great biological interest which have been reviewed in detail by Hawking and Thurston (1951). Work with species in dogs and monkeys has shown that when not in the peripheral blood, some 80 per cent of the microfilariae become concentrated in the lungs and very few occur in other parts of the body. The microfilariae seem to pass through the lungs without injury to the tissue in any way. How the microfilariae maintain themselves in the lungs during the non-migratory phase is a mystery; they may become coiled to hinder their passage through the capillaries.

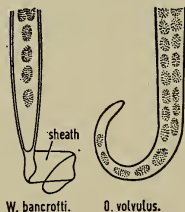


FIG. 149. Posterior end of a sheathed and an unsheathed microfilaria (after Blacklock and Southwell, 1954).

A number of hypotheses have been put forward, and these are briefly commented on below:

(a) The microfilariae in the peripheral blood are those of a new brood which are destroyed by the reticulo-endothelial system before the next brood appears.

*Comment.* This theory can readily be shown to be untenable, for on killing the adult worms by drugs such as neostibosan, the microfilariae can survive for several months.

(b) The periodicity depends in some way on the habits of the insect vector: if the vector is nocturnal in biting habits, then the microfilariae will exhibit nocturnal periodicity; if the vector is diurnal in biting habits, then the larvae will be either diurnal in periodicity, or be always present in the blood. It

has been suggested that there exists a specific chemotaxis which causes the microfilariae to migrate towards the saliva bite of the insect host.

*Comment.* Whereas it is true that the presence of the microfilariae in the blood stream is closely correlated to the biting habits of the vectors, there is no direct physiological evidence that the microfilariae are subject to chemotaxis of this type.

(c) The habits of the microfilariae are related in some way to the general waking and sleeping habits of the host.

*Comment.* This is generally true, for if these habits are reversed, as on a journey half way around the earth, the periodicity slowly follows suit. It can be shown experimentally that, at least in the case of *W. bancrofti*, microfilarial periodicity is orientated to the sleeping and waking habits of the host and not merely to darkness and light. It seems likely that there is some chemical change in the blood which promotes both the sleepiness of the host and the liberation of the microfilariae in the evening. In the morning, the converse change must take place. Hawking and Thurston (1951) found that the injection of an anaesthetic such as nembutal into monkeys during the daytime, frequently (but not always) produced considerable liberation of microfilariae into the peripheral blood.

The phenomenon is at present regarded as an attempt on behalf of the organism to satisfy its two main biological requirements: optimum survival and transmission. As a teleological explanation, the lung may be regarded as the most favourable site in the body for the microfilariae to exist, but if they remained there always, they would never come in contact with the insect vector and transmission to new hosts would be impossible. Migration to a possible transmission site (i.e. to the peripheral blood stream) is thus essential for the continuing of the species, but nutritional requirements must also be satisfied by spending part of their life in the most favourable environment, which is the lungs (Hawking and Thurston, 1951).

### 29.17 Superfamily Spiruroidea

The Spiruroidea contain a large assemblage of worms which are parasitic in a variety of environments, such as the alimentary canal, respiratory system, eyes, nasal cavities and sinuses of vertebrates. Their life cycles, where known, require an intermediate host. The body form shows great variation and can be slender and filarial-like, thick and solid-looking or even spherical. The majority have a single pair of lateral lips or an additional pair of dorso-ventral lips, but never three or six lips. The tail is spirally coiled and the eggs are thick-shelled.

#### *Genus Gongylonema*

Species of this genus are parasites in the alimentary canal of rodents and domestic animals in tropical and sub-tropical countries. In Great Britain they have only been found in mice.

The best known species is *Gongylonema* (= *Spiroptera*) *neoplasticum*, which occurs in the tongue, oesophagus and forestomach of rodents. In these sites, it was thought to

induce malignant lesions and a long programme of research by the Danish worker Fibiger and his co-workers in the early part of the century supported this view. Recent critical experiments (Hitchcock and Bell, 1952) have entirely exploded this view and have shown that true malignancy cannot be produced by this nematode. The so-called 'malignancy' described by the earlier workers has been found to be associated with the fact that the rats used in the original experiments were unwittingly kept on a vitamin A-deficient diet, a classical example of the traps inherent in biological experiments. The question is further discussed on p. 369.

The intermediate hosts of *G. neoplasticum* are the cockroaches, *Periplaneta americana* and *Blattella germanica*, in which active larvae may be found encysted in the leg muscles some two to three months after ingesting faeces with eggs.

#### Genus *Habronema*

There are three relatively common species, *H. muscae*, *H. megastoma* and *H. microstoma*, all of which occur in the stomach of equines in nodules or tumors. Hatched larvae are passed out with the droppings and become ingested by fly maggots. The infective stage is reached by the time the adult fly emerges. The horse is infected either by direct ingestion of the fly or by larvae escaping from the proboscis of flies settling on the horse's lips. The stable fly, *Stomoxys irritans*, acts as the host for *H. microstoma*, and *Musca* spp. for *H. megastoma* and *H. muscae*.

#### Genus *Thelazia*

These are filiariid-like worms which live in the conjunctival sac, lachrymal ducts or nasal cavities of animals and sometimes man. The worms move across the eye intermittently and may injure the conjunctiva. The best known species are *T. callipaeda*, *T. rhodesii*, *T. gulosa* and *T. lachrymalis*. The intermediate hosts are various species of flies of the genus *Musca*, the first larva being picked up from the eye-secretion of cattle by the flies.

### 29.2 Sub-order 7: Camallanata

These are usually referred to as the 'dracunculoid' worms, after the best-known genus *Dracunculus*. They were formerly included with the Filarioidea. They are long filariform worms in which the mouth is simple or with lateral jaws. They are parasitic in the connective tissue or coelom of vertebrates. The life cycle usually involves a copepod host.

#### *Dracunculus medinensis*.

The female worm is known as the 'guinea worm' and is probably the 'fiery serpent' referred to by Moses (Numbers 21); it has been known since early recorded history. *Geographical distribution*. Widely distributed in tropical Africa, Arabia, Dutch East Indies and India.

*Morphology*. The anatomy of the female is best known, the male remaining virtually unknown until 1936. The head bears a chitinous shield on which are six papillae (Fig.

150). The cuticle is smooth and milky-white. The alimentary canal atrophies in gravid females and the cavity becomes filled with the uterus packed with larvae. The females measure up to one metre in length. The males range from 12–29 mm with a conical tail and ten pairs of genital papillae.

*Habitat.* The adults occur in the subcutaneous tissues particularly those of the ankle and foot, arms and shoulders.

*Life cycle* (Fig. 151). The site of copulation is unknown, but the males disappear rapidly after the process and the females, when ripe, migrate to the superficial layers of the skin, especially to those regions liable to come in contact with water. Here a substance is

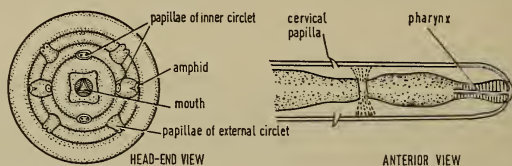


FIG. 150. *Dracunculus medinensis*—anatomy of anterior end of female (partly after Moorthy, 1937).

secreted which causes a blister to arise directly over the anterior end of the body where it has pierced the lower layers of the skin. The blister bursts eventually and a small, shallow ulcer is formed. On contact with water, the uterus is projected out of the ulcer cavity, and a cloud of milky-white secretion, containing hundreds of active larvae, is released. When the host steps out of the water, the exposed end of the uterus dries and shrivels and so blocks the release of further larvae.

The whole has been described as 'one of the neatest adaptations in behaviour in all the realm of biology, enabling an unmeditative, burrowing worm to give her aquatic *Cyclops*-inhabiting offspring a fair chance in life, even on a desert'. (Chandler, 1955.)

The food reserves of the released rhabditoid larvae are small but sufficient to enable them to survive in water for several days. If ingested by an appropriate species of *Cyclops*, they break through the soft mid-intestine wall and come to lie in the body cavity. Here the usual two moults are completed, and larvae become infective in about three weeks. Human infection is brought about by accidentally taking in infected copepods in drinking water. Under the stimulus of the gastric juice, the larvae become active, penetrate the gut wall, migrate through the tissues, moult twice and come to lodge in the viscera, or subcutaneous tissues. As a tissue site is a poorly nutritive environment, growth and development of the worm is slow and nearly a year is

required before sexual maturity is reached and female worms are ready to migrate to the skin and release their larvae.

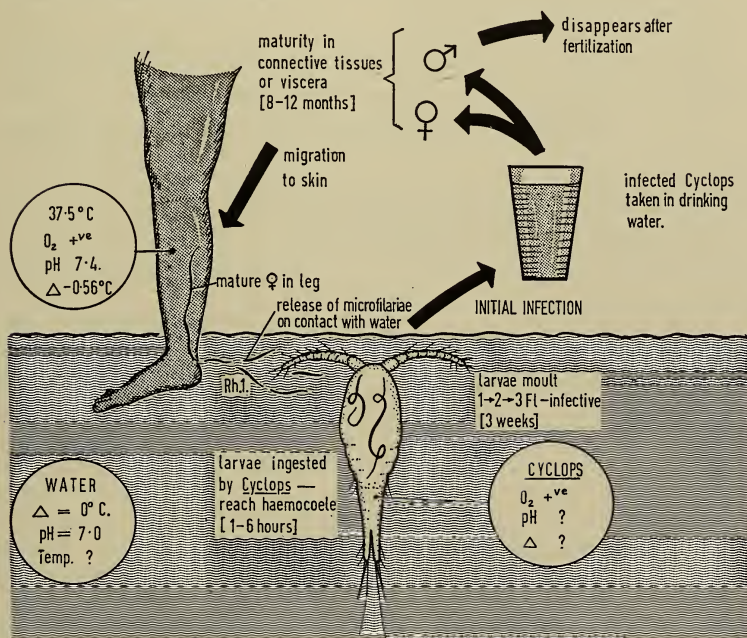


FIG. 151. *Dracunculus medinensis*—life cycle and some of the biological factors relating to it (original).

The disease caused by this parasite in humans is called *dracontiasis* or *dracunculosis*, and the centuries-old remedy has been to remove it by gently rolling the worm daily around a small stick and slowly pulling it out of the skin. With a few precautions, this method is still in use.

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## CHAPTER XXX

# PHYSIOLOGY OF NEMATODES

### 30.1 Chemical Composition

Most of the work on nematode biochemistry has centred around the large pig species *Ascaris lumbricoides*. Fairbairn (1957) has given a valuable review of the biochemistry of this species. The earlier literature is reviewed by Hobson (1948). The rodent trichostrongyle, *Nippostrongylus muris*, has also received some attention. As repeatedly emphasised throughout this text, data for the chemical analysis of parasitic worms are of little value unless related to the nutritional condition of the host at the time of autopsy. The composition of carbohydrates in particular is subject to rapid decline during starvation of the host, so that fluctuation between different specimens of the same species is to be expected.

**Carbohydrates.** As in trematodes (p. 212) and cestodes (p. 271) the main polysaccharide is glycogen closely resembling mammalian glycogen. In *Ascaris* and *Parascaris*, the molecular weight is approximately  $9 \times 10^6$  and the probable chain length 12–13 glucose units. Most of the glycogen is concentrated in the muscles but it is widely distributed in other tissues. The non-reducing disaccharide, trehalose, has also been isolated from *Ascaris* eggs, and a glucosazone-forming hexose has been found in *Ascaris* haemolymph (Rogers, 1945). Many nematode tissues contain a reducing sugar which is probably glucose.

**Proteins.** The proteins of nematodes are of exceptional interest but have been studied by modern analytical methods only in a few instances. The total protein in *Ascaris* makes up 8–9 per cent of the wet weight and the amino-acid composition generally resembles that of its host. The haemolymph of *Ascaris* contains 4.9 per cent proteins of which 2.8 per cent are albumins and 2.1 per cent globulins. The fibrous proteins of the cuticle have been most thoroughly studied but complete agreement has not been reached on their composition.

The layers of the cuticle are described on p. 300. The nature of the cortex is obscure although some workers have claimed it to be keratin on the basis of the X-ray pattern. Other workers believe

it to be collagen (Fauré-Fremiet and Garrault, 1944), although if this is true one would expect to find the amino acid hydroxyproline present in analyses and none has been reported (Savel, 1955). There is some evidence of quinone tanning in this layer.

In the fibrous layer which makes up the bulk of the cuticle, hydroxyproline has been found, and some workers claim that the X-ray pattern of the fibres is typical of collagen (Fauré-Fremiet and Garrault, 1944), although electron microscopy shows no typical collagen periodicities (Bird and Deutsch, 1958).

Many nematodes contain haemoglobin whose source varies with the species. In at least two well-documented cases (*Ascaris lumbricoides* and *Nippostrongylus muris*) the haemoglobin as adjudged by its spectrum is intrinsic and not derived from the host. In others (*Ascaris equinum* and *Eustrongylus ignotus*), the parasite haemoglobin gives a spectrum identical with that of the host, from which it is clearly derived. The haemoglobins of *A. lumbricoides* have been extensively studied by Davenport (1949) and Rogers (1949a and 1949b) and it is doubtful whether these pigments are of any value in the actual transport of oxygen.

Specialised protein granules are also found in the oocytes of nematodes and these play some part in the formation of the egg membranes although their precise function is not known. A further specialised protein termed *ascaridine* is synthesised in the testes and present in sperm; it appears to make an important contribution to the synthesis of ribonucleic acid in the egg of *Parascaris*.

**Lipids.** Whole *Ascaris* contains 1.1–1.8 per cent lipid (wet weight); all the usual fractions occur. The highest concentrations appear in the reproductive system (6.0 per cent) and the lowest in the haemolymph (0.3 per cent) (Fairbairn, 1957).

Free volatile and non-volatile acids occur in the haemolymph and in other tissues the acids occur as triglycerides. Analysis of the phospholipid fraction has yielded lecithins, cephalins and sphingomyelins with the lecithin preponderant (Rogers and Lazarus, 1949). The unsaponifiable fraction contains sterols and a high proportion of a waxy fraction termed 'ascaryl alcohol' and now known to consist of three closely related glucosides which have been called 'ascaroides'.

## 30.2 Nutrition

### 30.21 Adults

Very little detailed knowledge is available concerning the food requirements of zooparasitic nematodes, although the free-living forms have been extensively studied. The nature of such demands shows considerable variation during the life cycle. For example, an adult worm in the alimentary canal of a homoiothermic host producing enormous quantities of eggs will have greater nutritional demands than its free-living larva merely undergoing gradual growth and differentiation at air temperatures.

Carbohydrates and proteins form the main nutritional requirements, with fats playing a relatively unimportant role. A high carbohydrate diet is thus beneficial to the growth of most nematodes. Hormones, iron and certain unidentified growth factors may also be essential in most species. As is to be expected, a deficiency in the host diet may be reflected in the inhibition of the growth and reproduction of the worm. The extent and speed of this inhibition will depend, almost entirely, on the food reserves of the worm. Host starvation, for example, is reflected within 24 hours by a drop in the glycogen reserves of *Ascaridia galli*, followed by a fall in egg-production. Continued host starvation may result in the passing of the worm in two to four days. With the depletion of its main energy source, carbohydrate, a worm is no longer able to energise its muscles and so maintain its position in the intestine.

Deficiency in one or several nutritional components may provide unexpected results. Thus a deficiency of vitamin A in the host diet leads to decreased resistance to infection of rats to *Nippostrongylus muris* and dogs to *Ancylostoma caninum* and hence higher laboratory infections. Considerations such as these mean that it is seldom possible to state with certainty whether or not an observed effect is *directly* due to the nutritional deficiency under consideration. Such conclusions can only be reached on the results of carefully controlled experiments *in vitro*, where host reactions do not operate.

With a few exceptions, the food material of adult nematodes appears to be solid or semi-solid, and since the majority are intestinal parasites, this consists of semi-digested food and debris. It is unlikely that previously digested food is essential, however, as the following carbohydrate-, protein- and fat-splitting enzymes have been established in the gut of *Ascaris*: amylase, maltase, protease, peptidase, lipase and esterase. The first four enzymes have also been found in *Leidynema appendiculata*, and the same pattern is likely to be followed in other intestinal forms. The presence of alkaline and acid phosphatases in the gut cells suggests that nematodes, like vertebrates, utilise phosphorylation of monosaccharides for active absorption against a concentration gradient.

In the strongyles, blood and tissue form the main diet and many species, particularly hookworms, draw a plug of mucosa into their buccal cavity and suck blood and tissues. In some forms, the blood is almost continuously being drawn into the intestine and passed out through the anus. It has been calculated that a single *Ancylostoma caninum* is responsible for a loss of blood from the host of 0.4–0.8 ml per 24 hours. Both the plasma and corpuscles undergo at least partial digestion. The heart worms, *Dirofilaria immitis*, likewise feed on blood exclusively. Lungworms feed on the inflammatory exudate evoked by their own presence, as do some tissue forms.

In species with a capillary pharynx such as *Trichuris*, the lumen is not of sufficient diameter to permit the ingestion of solid food material and this nematode presumably takes in more or less liquid food.

To summarise, it may be concluded that most nematodes feed by means of their mouth, have a truly functional digestive canal and are capable of digesting at least carbohydrate, protein and possibly fat. There is no evidence that absorption of nutrients through the body wall can take place; this possibly occurs in *Dracunculus* (p. 346).

### 30.22 Larvae

The nematode egg contains sufficient endogenous reserves to enable a fully formed active larva to develop without the absorption of further nutriment. Thereafter, food is required to supply energy and materials for synthesis of new tissue. Nematodes are peculiar in one respect, since with a few exceptions there is *no* somatic cell multiplication after hatching in spite of very considerable increase in size. The major growth requirements of nematodes—prior to maturation—are thus for cytoplasmic rather than nuclear synthesis, and nucleic acids occur in nematodes in small quantities relative to the total tissue weight. In ovaries, the RNA/DNA ratio is about 4:1.

Free-living stages of nematodes (e.g. hookworms) must feed almost entirely on bacteria and such stages are readily cultured in faecal-charcoal cultures in which abundant bacteria are normally available. Axenic culture of these later stages has also been completed for a number of strongyles, namely *Ancylostoma braziliense*, *A. caninum*, *A. duodenale* and *Nippostrongylus muris* using complex semi-solid media such as embryo extract, liver or kidney homogenates (see p. 431).

Larvae with a tissue-migrating phase (e.g. *Ascaris*) presumably ingest sufficient blood or tissue during their migrations, to satisfy the growth requirements up to the fourth moult. It is significant that the most rapid growth phase does not take place until the intestine, with its abundant supply of food materials, is reached.

## 30.3 Respiration

As the life cycles of the majority of nematodes involve complex environmental changes, it is to be expected that marked differences between the respiratory metabolism of the different stages will exist. An egg, for example, may embryonate and hatch in an aerobic environment (moist ground), pass through its later larval stages similarly in an aerobic environment (tissues or lungs) and finally mature and produce eggs in the anaerobic environment presented by the gut. This pattern is reflected in the few species which have been studied in detail.

### 30.31 Eggs

Figures for the respiratory quotients so far investigated vary between 0.6 and 0.9. This fact, together with other evidence suggests that the energy for embryonic development is obtained mainly from the oxidation of fat, with carbohydrate and protein playing a lesser role. By a detailed analysis of the developing egg, it has been shown that a net decrease in the lipid corresponds with an increase in carbohydrate (Passey and Fairbairn, 1957). This suggests the synthesis of carbohydrate (trehalose and glycogen) from triglycerides. Respiration in all nematode eggs studied is inhibited by cyanide, carbon monoxide or hydrozoic acid, indicating dependence on a cytochrome system.

### 30.32 Larvae

The respiratory pattern of the nematode larvae which have been studied follows closely that of eggs in that it is essentially aerobic, but only a limited number of species has been studied and there may be exceptions to this generalisation. The R.Q. of infective larvae of *Haemonchus* and *Nippostrongylus* is about 0.7, a figure in keeping with morphological finding that these forms largely metabolise fat. On the other hand, tissue forms such as *Eustrongylus ignotus* (in muscles of fish) and *Trichinella spiralis* (in mammalian muscles) which have large glycogen reserves and a predominantly carbohydrate metabolism, have an R.Q. of 1.0 or over (Table 41).

TABLE 41  
RESPIRATORY QUOTIENTS OF VARIOUS PHASES OF  
NEMATODES, AT AN OXYGEN TENSION OF ABOUT  
160 MM. HG, AND IN THE ABSENCE OF SUGAR

(data from von Brand, 1952). Unless otherwise specified, values are given for 37–38° C.

Species	Respiratory quotient		
	Eggs	Larvae	Adults
<i>Haemonchus contortus</i> . .	*0.60–0.58	0.64	—
<i>Ascaris lumbricoides</i> . .	—	—	3.4
<i>Ascaridia galli</i> . .	—	—	0.96
<i>Nippostrongylus muris</i> . .	—	*0.73–0.66	0.69
<i>Trichinella spiralis</i> . .	—	1.1	—
<i>Syphacia obvelata</i> . .	—	—	1.1
<i>Litomosoides carinii</i> . .	—	—	0.44
<i>Eustrongylodes ignotus</i> . .	—	1.0	—
<i>Neoapectana glaseri</i> . .	—	—	*0.59

\* at 30° C.

### 30.33 Adults

Most of the work on adult nematodes has been carried out on ascarids, particularly the pig and horse species, and on strongyles. The  $Q_{O_2}$  of the common ascarids



is of the order of 0.4 but enormously higher in the strongyle *Nippostrongylus muris*, for which a figure of 6.8 has been obtained. Access to oxygen in these species must vary considerably. Large ascarids which lie in the lumen of the intestine will have access to little oxygen, whereas *Nippostrongylus*, with its anterior end embedded in the mucosa and sucking quantities of blood, may have access to substantial quantities. Horse and pig ascarids undoubtedly lead an essentially anaerobic existence, a conclusion confirmed by the fact that their oxygen uptake is entirely unaffected by cyanide, and cytochrome oxidase has not been detected in their tissues. They probably make use of flavine enzymes which react directly with oxygen to form hydrogen peroxide. On the other hand, respiration in *Nippostrongylus* is 40–60 per cent depressed by cyanide, indicating their dependence on the cytochrome system. This depression is even more marked in the case of *Neoaplectana glaseri* (p. 317), which is only a facultative parasite and essentially aerobic; its oxygen consumption is 96 per cent inhibited.

Although ascarids are probably essentially anaerobic forms, they are facultative rather than obligate anaerobes and utilise oxygen if it is offered to them. This utilisation of oxygen after an anaerobic period is common to free-living invertebrates also and has already been referred to as the 'repayment of an oxygen debt' (p. 218). The nature of this phenomenon is very imperfectly understood and the degree to which the 'debt' is repaid ranges from a small fraction to more than 100 per cent. It is possible that the repayment of the 'debt' only takes place when there is a sufficient accumulation of end-products of anaerobic fermentation in the tissues.

### 30.4 Carbohydrate Metabolism

Nematodes, like most helminth parasites, have a pronounced carbohydrate metabolism which has been extensively studied but which is still very incompletely understood. In the case of forms in anaerobic or semi-anaerobic environments, such as the intestine or bile ducts, this is not surprising, for carbohydrate offers the most convenient and most readily available source of energy under these conditions. What is emerging from recent studies is the fact that many parasites such as the filariid *Litomosoides*, which lives where oxygen is abundantly available (e.g. the tissues or the blood stream) and which could theoretically derive energy from the oxidation of proteins or fats, have a predominantly carbohydrate metabolism. However, this is of such a nature that the sugars are not completely oxidised to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  which means that it is of a fermentative type.

Large parasites such as *Litomosoides* and *Dracunculus* show a carbohydrate consumption per gram of tissue nearly forty times greater than that of small species such as *Eustrongyloides*, suggesting the existence of markedly different types of metabolism in different species. Part of this difference may be due to differences in maturity, as the



energy requirements of young forms undergoing organogeny are unlikely to approach those of adult forms producing many thousands of eggs a day.

The main reserves in nematodes are glycogen (p. 349), which is depleted rapidly under starvation conditions with the formation of fermentation acids and carbon dioxide. If glucose is present in the culture medium, the quantity of acids secreted is approximately doubled. The metabolic end-products in all species studied are remarkably similar, consisting for the most part of volatile fatty acids of which lactic, acetic, butyric, valeric and caproic have been identified with certainty. The major excretory product is valeric acid or its isomers, and in *Trichinella* this acid is responsible for more than 80 per cent of the acid produced. There is evidence that part of the usual glycolytic transformations of the Embden-Meyerhof scheme (Table 38) are followed; esterification of inorganic phosphate takes place but at some point a division into two 3-carbon, units takes place. The point of this division has not been determined. A further curious point in the metabolism of *Ascaris* is that the muscle in this species does not appear to contain appreciable quantities of the high-energy phosphate reserves (phosphagens) which are widely used by animals under stress.

The metabolism of trehalose, whose presence in nematode tissues has already been referred to (p. 349), has not been investigated.

Little is known about carbohydrate anabolism. Glycogen synthesis in *Ascaris* results from absorption of fructose, sorbose, maltose and sucrose, but not mannose or lactose.

### 30.5 Protein Metabolism

Most nematodes produce enormous quantities of eggs. A single *Ascaris lumbricoides* produces 200,000 daily, a single *Ancylostoma duodenale* 20,000 and a single *Haemonchus contortus* 5,000. It is thus evident that the group possesses remarkable powers of protein synthesis, being able rapidly to convert absorbed protein food material into eggs. Also, on account of the high lipid content of eggs, lipid synthesis must be highly significant. Yet little is known regarding the protein requirements and metabolism.

The end-products of nitrogen metabolism have only been studied in detail in a few species, but there is increasing evidence to show that the nitrogen metabolism is predominantly ammoniotelic.

This is a most interesting result and it suggests a basic nitrogen metabolism similar to that of free-living aquatic forms such as annelids, echinoderms, teleosts and reptiles (Rogers, 1955). In all these organisms, water is freely available to permit the ammonia concentration to be kept below a toxic level, and it may be concluded that similarly the nematodes can derive adequate water from the intestinal contents, since ammonia is highly toxic to nematodes.

Terrestrial animals, on the other hand, may suffer water shortage and have a ureotelic nitrogen

metabolism in humid environments or a uricotelic nitrogen metabolism in arid environments. On these grounds, therefore, it may be expected that the free-living stages of some nematodes, which may live under conditions of water shortage (although requiring at least a film of water), will exhibit uricotelism.

In *Ascaris*, 39 mg N per 100 g of wet weight per day is excreted, of which 79 per cent is ammonia, 7 per cent urea, 21 per cent polypeptides (Savel, 1955). In larval *Trichinella spiralis*, 2.8 mg N per 1 g of wet weight is excreted, of which 33 per cent is ammonia, 7 per cent volatile amine nitrogen, 20 per cent peptide nitrogen and 29 per cent amino-acid nitrogen; some eleven free-amino acids were identified (Haskins and Weinstein, 1957a, b, c). A similar pattern is found in *Ascaridia galli*, *Nematodirus spathiger*, and *N. filicollis*, the free amino acids occurring being almost identical to those excreted by *T. spiralis* (Rogers, 1952, 1955). Small quantities of urea have been reported in many cases, but uric acid is absent or present in trace quantities only.

### 30.6 Fat Metabolism

The fat metabolism has been little studied. Both lipases and esterases have been found in the gut of intestinal nematodes, but it is not known whether or not nematodes can absorb fatty acids from their intestinal contents. In larvae leading an aerobic life, such as those of *Nippostrongylus* and *Ancylostoma*, there is an evident drop in morphologic fat with age, suggesting that these larvae derive much of their energy from fat. Larval *Trichinella* similarly use fat aerobically *in vitro* as a source of energy.

In adult *Ascaris*, the lipid catabolism is limited, as evidenced by the fact that five days' starvation produces no appreciable fall. Nearly two-thirds of the total lipids occur in the female reproductive system, and the uterine eggs contain some 35 per cent lipids. Little is known regarding the mechanisms involved in this lipid synthesis.

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## CHAPTER XXXI

### ACANTHOCEPHALA

The Acanthocephala represent a group of worms parasitic in all classes of vertebrates but especially in fishes and birds. They show similarities both with the Platyhelminthes and the Nematoda, but no agreement has been reached on their phylogenetic position. They resemble Cestoda in lacking an alimentary canal. The chief diagnostic feature of the group is the presence of an invaginable proboscis armed with hooks from which the common name 'spiny-headed' worm is derived. They range in size from 1.5 mm to over 500 cm. The majority of species are small; the largest being *Macracanthorhynchus hirudinaceus* from pigs. The females are nearly always larger than the males. The intermediate hosts, in the few species where these are known, are arthropods, and paratenic hosts frequently occur. In their general anatomy, life history and habits they show a remarkable degree of uniformity.

#### 31.1 Available Species

In Europe, both larval and adult material is available. Adult male and female *Acanthocephalus ranae* (Fig. 152) are frequently found in the intestine of the toad *Bufo bufo*. The intermediate host of this species is the isopod *Asellus* sp.

Another useful laboratory species in Great Britain is *Polymorphus minutus* which is common wherever wildfowl or domestic ducks have access to lakes or streams inhabited by *Gammarus* spp. All three native species of the latter serve as intermediate hosts. A considerable practical advantage with *P. minutus* is that the larvae (cystacanths) in the gammarids take up a bright orange pigment, with the result that infected shrimps are easily recognisable. *P. minutus* has also been reported from N. America.

Other species available in Great Britain are *Echinorhynchus truttae* (adults in brown trout and larvae in *Gammarus* sp. but not so obviously pigmented as *P. minutus*), and *E. lucii* (adults in eels, larvae in *Asellus* spp.).

In the United States, *Neoechinorhynchus emydis* can usually be obtained from the gut of North American freshwater turtles. A good account of the anatomy of this species

has been given by Goodchild (1950). Another available species in N. America is *Moniliformis dubius* (= *M. moniliformis*) in the rat, and some biochemical work has been carried out on this species. The intermediate host is *Periplaneta americana*. Since both definitive and intermediate host can readily be maintained in the laboratory, this species is especially suitable for experimental work.

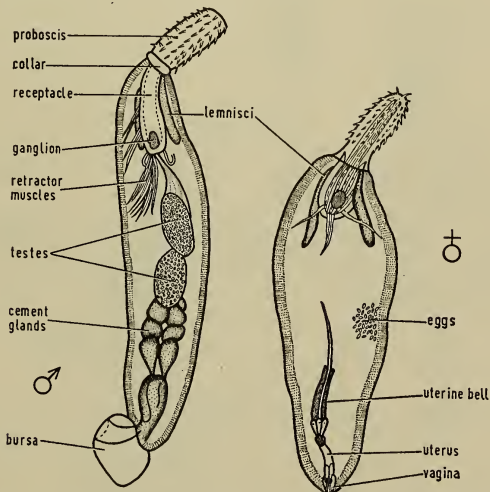


FIG. 152. *Acanthocephalus* sp.—morphology of adult male and female (after Yamaguti, 1935; Van Cleave, 1915).

### 31.2 General Account

#### 31.21 General Morphology

**Body.** The body consists of an anterior *presoma* with a characteristic spiny proboscis and a neck free from spines, and a posterior *trunk*. The spines on the proboscis are symmetrically arranged and of diagnostic value. The proboscis (and sometimes the neck) is usually retracted into a proboscis sac by being completely inverted, and the whole presoma is also retractile. Retraction is carried out by means of retractor muscles (Fig. 152).

The body wall consists of a syncytial hypodermis (or subcuticula) covered by a

cuticle. The hypodermis in primitive forms has only a small and fixed number of nuclei (6-20), which sometimes fragment into numerous nuclear pieces. Within the hypodermis run a series of vessels, the *lacunar* vessels, which serve to distribute absorbed food material. The hypodermis is confluent with two large organs, the *lemnisci*, which occupy

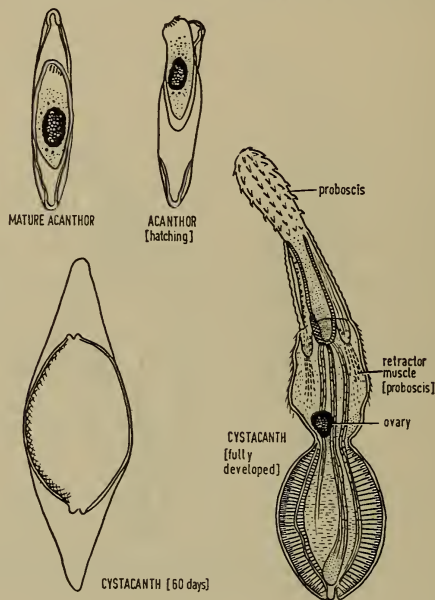


FIG. 153. *Polymorphus minutus*—morphology of developmental stages (after Hynes and Nicholas, 1957). Mature acanthor, from body cavity of female worm; acanthor, hatching in *Gammarus* gut; 60-day cystacanth, from body cavity of *Gammarus*; fully developed cystacanth, 24 hrs. after being fed to a duck, hind end still retracted.

lateral positions in the body cavity, extending beside and behind the proboscis sac. The lemnisci have a fixed number of nuclei which sometimes fragment. Their function is apparently to act as reservoirs for the lacunar fluid which is utilised in the retraction and protrusion of the proboscis.

*Digestive system.* This is lacking, as in cestodes.

*Excretory system.* Lacking, except in the order Archiacanthocephala, in which modified



protonephridial organs occur. These consist of flame bulbs and ducts which lead into a single duct or bladder (Fig. 154).

*Nervous system.* This is reduced to a ganglion in the wall of the proboscis sac and two nerve trunks which run posteriorly from the brain obliquely to the body wall. In the male, there is a pair of genital ganglia in the penis base.

*Nuclear constancy.* The group exhibits the interesting phenomenon of nuclear constancy or *eutely*. In a given species, the number of nuclei in the organs and tissues attained during larval development remain constant, except in the gonads. Some nuclei in the epidermis of some species fragment into chromatin material.

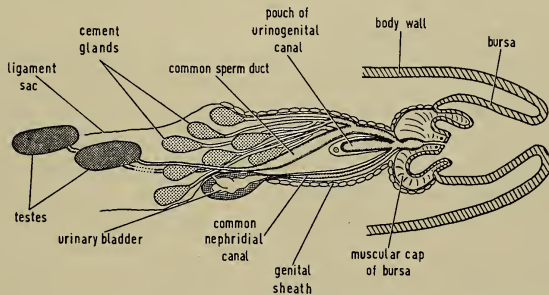


FIG. 154 Male reproductive system of *Hamanniella* (adapted from Kilian, 1932).

### 31.22 Reproductive System

*Ligament sac and ligament strand.* The ligament sacs are hollow tubes of connective tissue peculiar to the Acanthocephala; they enclose the gonads. In front, they are attached to the proboscis sac or the adjacent body wall, and posteriorly they connect to some part of the reproductive system. The number and arrangement of the ligament sacs varies in the different orders. Thus there are two (a dorsal and a ventral) in the females of the Archi- and Eoacanthocephala; but in the males of the same orders only the dorsal sac is present. In the Palaeacanthocephala, there is a single ligament sac in both sexes.

The *ligament strand* is a nucleated strand to which the gonads in both sexes are attached. This strand is believed to represent the endoderm and the body space between this and the body wall may be considered a *pseudocoel*.

*Male system* (Fig. 154). There are two testes attached to the ligament strand and enclosed in the ligament sac. In the Archiacanthocephala and the Palaeacanthocephala, there

occur *cement glands*, 6–8 in number, with ducts leading into the common sperm duct. In the Eoacanthocephala, however, the cement glands form a syncytial mass from which a duct enters a *cement reservoir* from which, in turn, ducts enter the sperm duct. The secretion of the cement glands seals or caps the female genital orifice after copulation.

Continuous with the ligament sac is a muscular tube, the *genital sheath*, which encloses the posterior ends of the sperm ducts, the cement glands and the protonephridial canals when present. There is a short penis which projects into a hemispherical or elongated *bursa*, a cavity made up of the in-turned body wall with a proximal muscular thickening, the *muscular cap*. The bursa can be everted to the exterior and is used in holding the female during copulation.

*Female system.* This shows a number of peculiarities. The ovary or ovaries (2) which are visibly intact only early in life, break up into *ovarian balls*, each consisting of a syncytium from which ovogonia are formed. These soon come to be free; in the Palaeacanthocephala the sac ruptures and they fill the body cavity. The ligament strands lead to a muscular organ termed the *uterine bell*. Posteriorly this opens into a *uterine tube*, bearing two *bell pouches*, which connects to a muscular *uterus* and a non-muscular *vagina* opening to the exterior. The vaginal aperture is guarded by a sphincter.

The uterine bell serves as a selective apparatus. Ovic larvae are taken in by the uterine bell and passed through the uterus to the vagina and so to the exterior. Immature eggs are rejected and returned via an aperture in the ventral bell to the dorsal pseudocoel. The uterine bell is continuous with the dorsal ligament sac in the Archi- and Eoacanthocephala. In the Palaeacanthocephala, the single dorsal strand connects to the inner side of the uterine bell.

*Copulation and fertilisation.* The male bursa is used for grasping the female and the penis is inserted into the vagina and sperm discharged. A secretion from the cement glands is then poured over the whole posterior tip of the female and the gonopore securely sealed.

*Structure of Eggshell.* The egg membranes consist of an outermost membrane, which is the fibrillar coat, the shell and the innermost membrane. The fibrillar coat consists of a keratin-like protein; the shell contains this protein probably reinforced with chitin. There is no evidence of quinone tanning (Monné and Hönig, 1954).

### 31.23 Life Cycle

The life cycles are known for only a few species; these are listed in Table 42. The intermediate hosts are insects (insect larvae, beetles or cockroaches) for those parasitic in land animals, and crustacea or molluscs for those in aquatic animals. Some

developmental stages of *Polymorphus minutus* are shown in Fig. 153. Hatching occurs within a few minutes of ingesting the eggs. The freed *acanthor*, armed with hooks and spines, rapidly penetrates the peritrophic membrane and pierces the gut to become free in the body cavity. It is now known as an *acanthella*. This slowly grows to the final larval stage or *cystacanth* which is enclosed in a delicate hyaline sheath secreted by the

TABLE 42  
SOME SPECIES OF ACANTHOCEPHALA WHOSE LIFE CYCLES ARE  
KNOWN IN DETAIL

Species	Definitive host	Intermediate host	Reference
<i>Moniliformis dubius</i> . . .	rat	<i>Periplaneta americana</i>	Moore (1946)
<i>Macracanthorhynchus hirudinaceus</i> . . .	pig	larvae and adults of	Kates (1943)
<i>Macracanthorhynchus ingens</i> . . .	raccoon	scarabaeid beetles	Moore (1946)
<i>Leptorhynchoides thecatus</i> . . .	freshwater fish	<i>Hyalella azteca</i> (amphipod)	De Giusti (1949)
<i>Neoechinorhynchus cylindricus</i> . . .	bass	<i>Cypris globula</i> (ostracod)*	Ward (1940)
<i>Echinorhynchus truttae</i> . . .	trout	<i>Gammarus</i> spp.	Scheer (1934)
<i>Neoechinorhynchus emydis</i> . . .	map turtle	<i>Campeloma rufum</i>	Hopp (1954)
		<i>Ceriphasia semicarinata</i> (freshwater snails)	
<i>Polymorphus minutus</i> . . .	duck	<i>Gammarus</i> spp.	Hynes and Nicholas (1957)
<i>Acanthocephalus ranae</i> . . .	amphibia	<i>Asellus</i> spp.	Van Cleave (1915)

\* utilises small fish as transport hosts.

larva. The cystacanths of *Moniliformis dubius* take about 7–8 weeks to develop in *Periplaneta americana*; those of *Polymorphus minutus* require about 8–9 weeks in *Gammarus* spp. There is some evidence of resistance of *Gammarus* spp. to infection by *P. minutus*, if a species of *gammarids* other than that used initially to infect the duck, was used (Hynes and Nicholas, 1958).

### 31.24 Classification (Based on Van Cleave, 1936).

Three orders are recognised:

**Order 1. Archiacanthocephala.** Hooks on proboscis either in long rows or in a few circles; spines lacking on trunk; main lacunar vessels dorsal and ventral; both dorsal and ventral ligament sacs present in female; cement glands separate.

e.g. *Moniliformis dubius*.

**Order 2. Palaeacanthocephala.** Hooks on proboscis usually in long rows; spines on trunk;

nuclei in hypodermis usually fragmented; main lacunar vessels lateral; ligament sac in female single; cement glands separate.

e.g. *Leptorhynchoides thecatus*.

*Order 3. Eoacanthocephala.* Hooks on proboscis usually in a few small circles; few nuclei (large) in hypodermis; main lacunar vessels dorsal and ventral; dorsal and ventral ligament sacs in female; cement glands syncytial.

e.g. *Neoechinorhynchus emydis*.

### 31.3 Physiology

The physiology of the group is very poorly known, but in general it appears to resemble that of cestodes rather than nematodes. In the absence of an alimentary system, food materials must necessarily diffuse through the body wall. It would be of interest to determine whether electron microscopy will reveal the presence of cuticular villi such as have been shown to be present in cestodes (Fig. 89).

*Chemical composition.* The chemical composition of *Macracanthorhynchus hirudinaceus* (von Brand, 1939) is as follows: water, 88.5 per cent; protein, 8 per cent; glycogen 1.1 per cent; lipids, 0.95 per cent; salts, 0.6 per cent; [percentage of fresh weight]. The composition of *Moniliformis dubius* so far determined (Laurie, 1959) is as follows: glycogen,  $6.74 \pm 2.24$  per cent; trehalose,  $1.83 \pm 0.81$  per cent; nitrogen,  $6.1 \pm 0.4$  per cent (males)— $7.7 \pm 1.3$  per cent (females) [figures in percentage dry weight]. As stressed in the case of cestodes (p. 269) and nematodes (p. 212), to be of real value these figures must be considered in relation to the diet of the host, for under adverse nutritional conditions considerable fluctuations will occur. Thus, starvation of the rat host for periods of 24–80 hours produced a marked decrease in both the polysaccharide content and the wet weight of *Moniliformis dubius* (Read and Rothman, 1958) and there was evidence of a diurnal fluctuation.

*Metabolism.* The only species investigated in detail, *Moniliformis dubius*, exhibits a strong fermentative type of metabolism similar to that of cestodes, even under aerobic conditions (Laurie, 1957, 1959). The main carbohydrate reserves are glycogen; trehalose, glucose, fructose, mannose and maltose can be utilised under aerobic or anaerobic conditions, and galactose under anaerobic conditions. Alkaline phosphatase has been detected in the outer layer of the subcuticula of the trunk region, presumably associated with absorption or secretion from the body surface (Bullock, 1958).

During aerobic fermentation acetic, lactic and formic acids are produced in proportions depending on the substrates used.

The above results suggest that Acanthocephala in general may have a metabolism

similar to that of cestodes based on the Embden-Meyerhof scheme of reactions. Experimental work in this group is, however, exceedingly meagre.

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## CHAPTER XXXII

### THE HOST:

### REACTIONS AGAINST THE PARASITE

When a host is invaded by a parasitic organism, it can respond by bringing into action defence mechanisms which, broadly speaking, are divided into two groups:

- (a) tissue reactions;
- (b) immunity or resistance.

These two types of reactions are closely inter-related. *Tissue reactions* tend to be localised in the immediate site of parasite invasion. They usually appear more rapidly than immune reactions and often disappear after the invading organism has left or has been destroyed. *Immunity* (resistance) is the result of a more generalised effect usually originating in organs or systems far removed from the site of infection. It may be considered a reaction of the whole body in attempting to dispose of foreign material. This type of reaction develops more slowly than a tissue reaction but generally persists for a longer time; indeed, it often persists throughout the life of the host. Immunological mechanisms are becoming of increasing importance in parasite biology and will be considered in some detail later. The terms *resistance* and *immunity* as used by most modern authors are essentially synonymous and will be generally used as such here. Theoretically, the word 'resistance' is a more precise term implying, as it does, that the reactions developed by the host may not be completely successful in repelling invading organisms. The term 'immunity' suggests complete freedom from the organisms.

#### 32.1 Types of Host Tissue Reaction

These are fully discussed in text-books of pathology, and will only be briefly dealt with here. In general, reactions under this heading are provoked by any type of foreign invader, whether living or non-living. They represent a combination of direct damage to the host tissue and a reaction by the tissue to mechanical irritation or to the released metabolic products of the invader.



The main evidence of damage to the tissue cells is cloudy swelling of the cytoplasm, fatty degeneration or total necrosis of cells. As a result of damage to the host tissue there is an acute *inflammatory reaction*. This shows itself as a swelling of the tissue (*oedema*), brought about as a result of localised dilation of the capillaries (*vasodilation*) and the outpouring of plasma and leucocytes into the affected area. If the invading organism is small, complete destruction may occur by phagocytosis and the inflammatory reaction will resolve itself, but where chronic inflammation occurs, due to the persistence of the invader, tissue changes occur. New capillaries grow inward into the affected region, and at their tips fibroblasts proliferate to form eventually a wall of fibrous tissue around the parasite. Within this capsule, macrophages may remove damaged tissue and eventually the parasite itself may be destroyed. A typical example of a reaction of this type is that against a larval helminth (e.g. a cercaria or a plerocercoid) which encysts beneath the skin of an intermediate host (e.g. the trematode *Cryptocotyle lingua* (p. 180) in marine fish).

The capsule formed in these cases is called a *host tissue capsule*. In several trematodes, the formation of a capsule of this kind, in addition to the cyst formed by the parasite, is accompanied by the deposition of pigment, usually melanin, resulting in the familiar 'black spot' of fish. Similar cysts are formed against many nematodes (e.g. *Trichinella spiralis*). In some cases, if capsules persist for prolonged periods, calcification may occur.

*Induction of abnormal growth.* One of the most interesting features of tissue reactions often associated with the presence of parasites is a change in the growth pattern of parasitised tissue. This change of pattern may be reflected in a number of ways (Lapage, 1951), as follows.

(a) *Hyperplasia*, that is an increase in the rate of cell division. In this condition, the cells increase in number but not in size. This is seen frequently during repair, when the reparative processes have been carried out in excess of the actual requirements. It is often produced as the result of irritation (e.g. the liver of a rabbit infected by *Eimeria stiedae*; p. 76).

(b) *Metaplasia*, which is the transformation of one tissue type into another. It is not commonly associated with the presence of animal parasites, but is known to occur, for example, in the lungs of mammals infected with the fluke *Paragonimus westermanii* (p. 181).

(c) *Neoplasia*, which is the growth of a new kind of tissue from existing cells, and such growths are commonly referred to as *tumours*. Any definition of a tumour can only be regarded as temporary and provisional. 'A neoplasm or tumour may be defined as a new growth arising from pre-existing tissue, independent of the needs of the

organism, and subserving no useful purpose, but on the contrary, often acting deleteriously. It is autonomous, i.e. it follows its own line of growth, and is not regulated by those governing the tissues of the body in which it is growing. . . . the life history . . . of a neoplasm appears to have no typical termination, the tumour cells seeming to be endowed with the power of continuous, aimless and unlimited proliferation.' (Beattie *et al.*, 1948). A malignant tumour is popularly referred to as a 'cancer'.

'The term cancer is a convenient label or pigeonhole for a number of pathological tissue conditions exhibiting certain common characteristics, but all unique in certain other respects. Indeed, it is not a disease at all, but an assemblage of many different diseases which have certain symptoms in common. A century ago, medical men spoke of "fever" as a disease. Now they no longer do so. Something of this sort is happening with cancer during the present century.' (Huxley, 1958).

In medical terminology, however, the term 'cancer' is used synonymously for a *carcinoma*, a malignant tumour of epithelial tissue. The term *sarcoma* refers to a malignant tumour of connective tissue.

TABLE 43

PARASITES BELIEVED TO BE ASSOCIATED WITH TUMOUR  
FORMATION (NEOPLASIA) IN MAMMALS

Only in the case of those marked \* has the relationship been unequivocally established (data mainly from Schwabe, 1955).

Group	Parasite	Host	Site of tumour
Protozoa . .	<i>Eimeria stiedae</i>	rabbit	liver
Trematoda . .	<i>Schistosoma mansoni</i>	man	intestine
	<i>Schistosoma haematobium</i>	man	bladder
	<i>Schistosoma japonicum</i>	man	intestine
	<i>Paragonimus westermanii</i>	tiger	lung
Cestoda . . .	<i>Clonorchis sinensis</i>	man	lung
	* <i>Cysticercus fasciolaris</i>	rats	liver
	<i>Echinococcus granulosus</i>	man	lung
	<i>Gongylonema neoplasticum</i>	rat	tongue
Nematoda . .	* <i>Spirocerca lupi</i>	dog	oesophagus

It is significant that helminth parasites are amongst the very diverse agents associated with the production of neoplasia; the recent literature has been reviewed by Schwabe (1955). A number of species, listed in Table 43, have been incriminated. Of these, only the larva of the cestode *Hydatigera taeniaeformis* (*Cysticercus fasciolaris*) in the liver of rodents, and the adults of the nematode *Spirocerca lupi* in the oesophagus of dogs have been unequivocally shown to be associated with sarcoma formation in these sites. The other cases listed are doubtful ones. The presence of *Schistosoma mansoni*, *S. japonicum* and *S. haematobium* has long been associated with cancer in man. Yet the

evidence is not clear-cut and it has never been demonstrated that the incidence of cancer is greater in a bilharzial population than in a similar but uninfected population.

Hepatic sarcoma in the liver of the rat induced by the presence of *Cysticercus fasciolaris* has received much attention since its initial discovery by Borrel in 1906. To date more than 7,500 cysticercus tumours have been experimentally developed. The majority of these have been sarcomas, but some have been bone-forming tumours and a few have been of other histological types. In general, malignant tumours arise only following an infection of from 250 to 675 days duration. The tumours appear to arise not from the liver tissue itself, but from the fibrous capsule formed by the host around the larva. Attempts to isolate a specific active agent from *Cysticercus fasciolaris* have been unsuccessful (Dunning and Curtis, 1953); there is some evidence that it may be associated with the calcareous corpuscles normally present in the body fluid of cysticerci. (Dunning and Curtis, 1946.)

The extraordinary difficulties inherent in attempts to obtain unequivocal results in experimental work on tumour formation are reflected in the case of *Gongylonema neoplasticum* (p. 344). This nematode occurs in the tongue, oesophagus and forestomach of rodents. In these sites, it was thought to induce malignant tumours and an intensive research programme was carried out on this organism especially by Fibiger and his co-workers. Recent critical experiments (Hitchcock and Bell, 1952) have shown that, although this nematode can act as a chronic irritant, it does not induce malignancy. It was shown that the so-called malignancy described by early workers was related to the unintentional use, in the original experiments, of Vitamin A deficient diets for the experimental rats used. The parasite was first encountered in rats in a sugar refinery where the abundance of sugary food available resulted in the natural diet being a vitamin A deficient one, although this was unrecognised at the time. When the infections were established and maintained in laboratory rats, it happened that a vitamin A deficient diet consisting of white bread and water (not milk) was again used. Examination of the records of the millers suggests that the bread used in Norway at that time was deficient in vitamin A. Thus, by a series of coincidences, the abnormal diet conditions occurring in the sugar refinery were essentially reproduced.

### 32.2 Immunity (Resistance)

The basis for resistance to animal parasite infections is essentially the same as that for viruses, bacteria or non-living materials and it is important to have some understanding of the basic concepts established in this field.

If a parasite has an opportunity to become established in a host and fails to do so,

never having previously come in contact with this particular host, it is said in a general way to possess a natural resistance to infection by this species of parasite. The mechanisms preventing the establishment of a parasite in a host may be extremely complex and frequently difficult to define in precise terms. In this respect, another term, 'susceptibility' must also be considered and it is important to distinguish between susceptibility and resistance. These terms, which are discussed in detail by Read (1958), may be defined as follows:

*Susceptibility* refers to that state in which a host is theoretically capable of being infected by a parasite and implies that there are no adverse physiological conditions which would eliminate a parasite before it had an opportunity to become established in the host.

*Resistance* may be defined as a physiological response by the host to a previous or present contact with the parasite, the nature of the response being such that it is directed against the establishment and survival of the parasite.

*Susceptibility.* When considering the susceptibility of a host, it must be borne in mind that although a host may be theoretically susceptible, the behaviour pattern may never normally permit the potential host and parasite to establish contact (see p. 371).

Of all the factors affecting susceptibility, host diet has perhaps the most pronounced effect. It is clear that a host fed on a diet deficient in some factor or factors necessary for the growth and development of a particular parasite will become insusceptible to infection to that parasite. If such a change in diet occurs naturally it could be more properly considered to be a case of behavioural resistance (see below). For example, with changing seasons the diet of a host may vary considerably. Beaver (1937) noted that pigeons become refractory to infection with the trematode *Echinostoma revoltum* when fed on a laboratory diet of yellow corn, wheat, rice and oats. Other instances are known, especially with protozoans, where the host diet may have a marked effect on the establishment and growth of the parasites (p. 64).

### 32.21 Natural Resistance

If a susceptible host comes in contact with a parasite, yet fails to become parasitised, it exhibits natural resistance to the parasite. The following types of natural resistance may be recognised:

- (a) Physiological resistance;
- (b) Behavioural resistance;

but it is sometimes difficult to decide into which category any particular example may fall.

A third category, *genetical resistance*, is sometimes included but it can be argued that since physiological properties (e.g. stomach pH) and (to some extent) behavioural patterns are gene controlled, the above two types of resistance are basically types of genetical resistance. A simple example of gene controlled resistance is that of *Culex pipiens*, individual specimens of which are completely resistant to *Plasmodium catheherium* (p. 82); the resistance in this case behaves as a Mendelian dominant.

(a) *Physiological resistance*. This type of resistance is due to some aspect of the host physiology being incompatible with that of the invading parasite at some stage in its life history. A well-known case is that of *Ascaris lumbricoides* (p. 317) of which the pig and human strains are virtually morphologically identical (but see p. 318) and yet infective larvae from the pig species will not develop to maturity in man and vice versa. Resistance in this instance is incomplete, since larvae from the pig species can undergo an early migrating phase in man, as can those of the human species in pig. Again, in order that a cyclophyllid larva may become established, its scolex must excyst and evert in the intestine. Excystment may be affected by a number of factors, such as priming effect of gastric juice, excitatory effect of bile salts, denaturing effect of bile salts on cyst proteins, proteolytic effect of trypsin and effect of temperature (Read, 1958). It has been found that certain species of worms will require interacting factors for excystment and that the required combination may only occur in specific hosts. Also, in the case of pseudophyllids, coracidia are killed by the digestive juices of some copepods and not by others; clearly, only the latter can act as intermediate hosts. Many other examples of a similar nature are known and there seems no doubt that physiological resistance is one of the most important factors operating in the determination of host specificity.

(b) *Behavioural resistance*. It is well known that many parasites have broad host spectra. This is especially true of helminths but less true of parasitic protozoans which show more restricted host specificity. The example of the avian cestode *Diphyllobothrium dendriticum* (which can mature in a rat) has already been quoted (p. 245). An organism with an unusually broad definitive host spectrum is the heterophyid *Cryptocotyle lingua* (p. 179) which is naturally an intestinal parasite of coastal birds but which will develop in many laboratory mammals such as rats, cats and guinea pigs. Ecological considerations are such that cats and guinea pigs never naturally come in contact with the fish intermediate host so that although they are theoretically 'susceptible' they are in practice resistant on account of their behaviour. However, it is possible that an occasional dockland cat becomes infected by scavenging infected fish. Wild rats, on the other hand, are well known for their scavenging habits and probably act as reservoir hosts in nature.

The borderline between 'behavioural resistance' and 'behavioural insusceptibility'



is understandably slight and a change in behaviour may render a normally susceptible host resistant. Thus the cestode *Hymenolepis nana* will develop successfully in the grey squirrel (*Sciurus carolinensis*) in the laboratory, if the latter is fed on dog biscuits, and it will develop more rapidly to a larger size and live longer than in its natural host, the mouse. Yet attempts to introduce *H. nana* artificially in a wild population of squirrels have failed. Likewise, attempts to infect laboratory-kept squirrels fed on natural diet have also failed, suggesting that the natural feeding habits of squirrels do not produce an intestinal environment suitable for establishment and growth of *H. nana*.

The view has also been put forward (Read, 1958) that sociopsychological stresses such as crowding may produce physiological changes in animals resulting in the lowering of the tissue responses related to the development of resistance. Such stresses stimulate the release of the adrenocorticotrophic hormone from the pituitary, resulting in increased production of adrenal glucocorticoids which in turn are believed to decrease the organism's immune responses. They also result in an increased rate of adrenalin secretion. It is thus possible that increased population density may result in a general lowering of the resistance of the population; perhaps, in extreme cases, having the effect of eliminating the less resistant with the consequent survival of the more resistant.

### 32.22 Acquired Immunity

Immunity may be acquired in two ways:

- (a) naturally, as a result of a natural infection acquired during the normal life cycle of an animal;
- (b) artificially, as a result of the deliberate introduction of the live or dead organism, or of an extract of it; this process being known as *vaccination*.

The general basis for acquired resistance to animal parasites is essentially the same as that for viruses, bacteria or non-living materials, study of which is broadly referred to as 'immunology'. The term 'serology' denotes that branch which deals with the manifestations of the immune reactions in sera, but through common usage, the terms 'serology' and 'immunology' have become virtually synonymous.

The basic principles of immunology are covered in detail in text-books such as those of Rafell (1953), Boyd (1956) and Cushing and Campbell (1957), but certain basic concepts will be considered in detail later. Immunology of parasitic helminths in particular are discussed by Culbertson (1941).

*Antigens and antibodies.* If foreign material (living or non-living) introduced into an



animal body causes a response, which characteristically is manifested by the ability of the body fluids, and sometimes the cells, to react with the provoking substance, it is termed an *antigen*. By far the greatest number of antigens are proteins, but other substances, especially polysaccharides, may also serve as antigenic materials. A molecule must have a minimal molecular weight of about 10,000 and its surface configuration must have a repetition of certain chemical groupings in order to act as an antigen. It is against these groupings that the specific antibody response is directed, and they

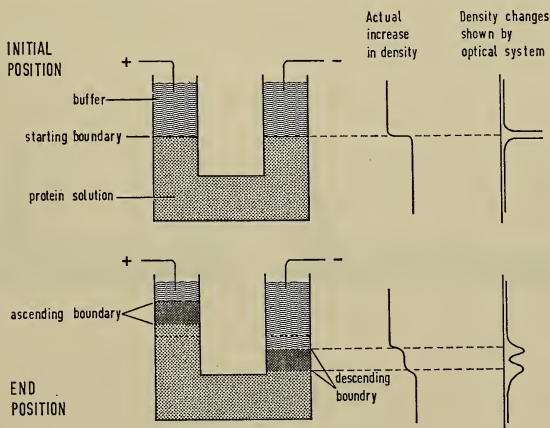


FIG. 155. Free-boundary electrophoresis of a system containing two types of proteins possessing different electrophoretic mobilities (adapted from Cushing and Campbell, 1957).

determine the specificity of the antigen molecule. The acid radicle appears to be the most important group constituent in determining antigenic specificity. Some substances, termed *haptens*, are able to act as antigens only when combined with 'carrier' molecules. The distinction between haptens and complete antigens may not always be sharp. In the case of bacteria and viruses, the body substance is the primary antigen, but recent studies with helminths and protozoans have shown that metabolic products such as enzymes or hormones, can also serve as antigens.

Introduction of an antigen results in the appearance in the blood, tissue fluids and sometimes the cerebrospinal fluids and certain cells, of a soluble protein entity termed *antibody*. Antibody has the property of combining specifically with the antigen which

provoked its production, *but with that antigen only*, so that the molecular structure of antibody is in some way related to that of antigen. Available evidence suggests that it is the reticulo-endothelial cells, particularly in the lymph nodes, which form antibodies. The rate at which antigens stimulate antibody formation varies considerably and is usually measured in terms of days. In rare cases, antibody may be formed within 40 minutes or less (Taliaferro, 1958).

*Structure of antibodies.* The protein in plasma may be separated by various physical and chemical procedures into two major fractions, *albumin* and *globulin*, present in a ratio of about 1.5-2:1. Electrophoresis (Fig. 155) is commonly employed. The globulin fraction may be further divided into *five* components: two  $\alpha$  globulins, two  $\beta$  globulins and a  $\gamma$  globulin (Fig. 156). Antibodies have been primarily identified

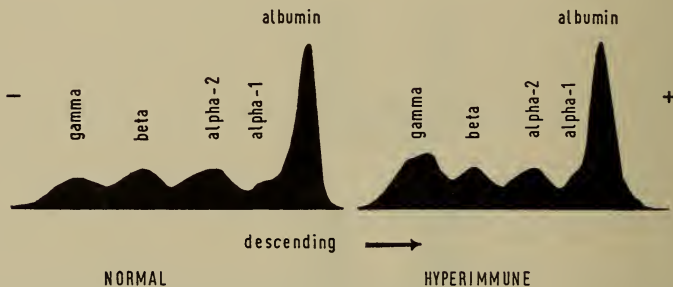


FIG. 156. Common electrophoretic descending boundary produced by normal and hyper-immune sera (after Cushing and Campbell, 1957).

with the  $\gamma$  globulin fraction, but it is becoming evident that they can be associated with the other globulin fractions also. With rare exceptions, most types of antibodies have a molecular weight of about 160,000.

The method of formation of antibodies is one of the most controversial problems in immunology. Detailed consideration is beyond the scope of this book but the position may briefly be summarised as follows. There are three main views: (a) the direct template theories, (b) the indirect template theory, and (c) the elective theories.

The direct template theories postulate that antigen enters the area of serum protein synthesis in some way and interferes with the arrangement of polypeptide chains into globulin molecules. The globulin thus takes on a configuration, part of which is complementary to one of the determinant groups of the antigen. The indirect template theory (of Burnet and Fenner) postulates that antibodies are replicas of adaptive enzymes, the production of which is stimulated by the presence of antigens to which an animal is exposed after birth. The elective theories postulate that an antigen merely has the role of stimulating the proliferation of a corresponding but *pre-existent* antibody. A much discussed modification of this view is the clonal selection theory of Burnet (1959) which postulates that before lymphoid cells reach maturity large numbers of variants arise, each capable of producing only one or two types of gamma globulin. When antigen gains access to the tissues, it combines with the cells which produce gamma globulin of the complementary configuration, and stimulates these cells to multiply; the level of the appropriate gamma

globulin is thus built up. According to this view, at birth an animal already possesses sufficient gamma globulin patterns to correspond with any antigenic determinant with which it may come in contact.

On any of these views, for an antigen to stimulate the formation of reacting antibody, therefore, the significant molecular pattern must not be destroyed or damaged. This explains why some biological antigens (e.g. bacteria) are only active in a living state, while others remain active after being killed by formalin treatment. Apart from its combining power with antigens, antibody globulin is indistinguishable from normal globulin and has the same chemical and physical properties.

Combination of antibody and antigen may produce clumping of antigen, or lysis of cells containing antigen, or more ready ingestion of such cells by phagocytes. These various reactions are discussed in detail later (p. 377).

Although in the majority of cases the appearance of antibody is related to the production of immunity in the host, the relationship is frequently not clear and in some cases the influence of antibodies on immunity appears negligible.

Serum of an immunised animal (containing antibodies) is termed *antiserum* and if some of this serum is injected into a non-immunised animal, this second animal also will exhibit immunity. Immunity produced by injection of antiserum is termed *passive immunity* since the recipient animal has not taken part in the development of the immune state. Duration of passive immunity is short, perhaps a few weeks, but depends partly on the amount of antibody injected. In contrast, the kind of immunity developed after the injection of antigen is termed *active immunity*, since the infected animal body is itself actively responsible for its production. The degree and duration of active immunity depends on a number of factors; in some cases it may last for years, in others it may wane sharply after the antigen has disappeared.

### 32.23 Antibody-Antigen Reactions

*General principles.* It is not possible to discuss in detail here the various hypotheses put forward to account for the reaction between antibodies and antigen. These are given in various treatises of immunology such as Raffel (1953). The most generally held view is that antibody and antigen possess multiple combining sites (Fig. 157) which provide bridging links so that a three-dimensional lattice structure is built up. It is believed that antigens always have many combining sites (polyvalent) and that antibodies usually have two (bivalent) but may only have one (univalent).

The proportions in which antibodies and antigens interact have a marked effect on the composition of the resulting precipitates. If a constant amount of specific antiserum is placed in a series of tubes and varying amounts of antigen added, *three* conditions may result (Fig. 157):

(a) *Excess antigen.* Resultant precipitate contains relatively more antigen than antibody (B. Fig. 157).

(b) *Immunological equivalence*. In which the combining sites of the antibodies and antigens are used to the full and there is virtually complete precipitation of antigen and antibody (C. Fig. 157).

(c) *Excess antibody*. Precipitate contains relatively more antibody than antigen. Excess antigen, however, tends to produce solubility of precipitate by removing antibody which would otherwise form lattice work (A. Fig. 157).

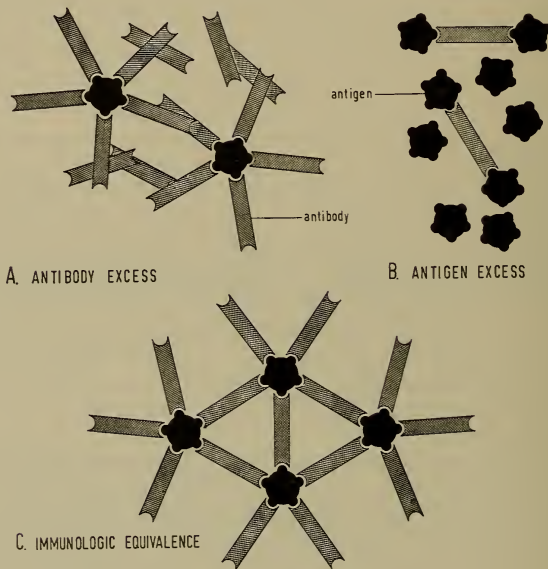


FIG. 157. Combination of antigen and antibody in different proportions (original).

*Visible reactions of antibody-antigen combination.* There are a number of major manifestations of antibody activity which may be visibly observed in a test-tube:

- (a) precipitation;
- (b) agglutination;
- (c) lysis;
- (d) opsonisation.

Any of these reactions may be used as a serologic test to determine the suspected presence of antibody (or antigen). Antibody concentration is expressed by the *titre* of a serum which is the number of antibody units per unit volume of undiluted serum. Detailed accounts of tests and procedures for measuring titre are available in standard immunological texts such as Kabat and Mayer (1948).

(a) *Precipitation*. The result of aggregation of *soluble* antigens by antibody action is observable under suitable conditions with suitable concentrations of antigen and antibody. The solution becomes turbid and an insoluble antigen-antibody complex precipitates out. The composition of this precipitate varies with the ratio of antigen and antibody components as already considered above.

(b) *Agglutination*. The basic mechanism of agglutination is essentially the same as that of precipitation except that the antigens are larger and either particulate or cellular (e.g. bacteria or blood cells). The ratio of antigen to antibody is, however, not as important in agglutination reactions as in precipitation reactions.

(c) *Lysis*

(i) *General*. Under certain conditions antibody may exhibit activity resulting in the lysis of cells acting as antigens. The reaction is not a general one but is limited to erythrocytes (the reaction in this case being termed *haemolysis*), a few bacteria and the vegetative forms of protozoans. The mechanism of lysis is not understood as it involves another component of normal serum termed *complement* which lyses cells after they have been sensitised by antibody.

Complement is a substance of mixed globulin composition, present in normal fresh serum. Its concentration varies in different species of animals but is constant for each species. Guinea pig serum contains more complement than most other sera and is extensively used in experimental work. Complement rapidly deteriorates after withdrawal from the body although loss of potency is inhibited by refrigeration.

(ii) *Complement fixation*. Complement will not combine with antigen alone and has only a loose affinity for antigen-antibody complexes and is actively absorbed by them from serum. This reaction is known as *complement fixation* and it is the basis of a sensitive and useful method for detecting antigen-antibody reactions. It finds its greatest application in bacteriology in the Wasserman test for syphilis, which may be briefly described as typically illustrating the principles involved.

The test serum is mixed with standard Wasserman antigen and fresh guinea pig serum as a source of complement. After allowing the mixture to react for some time, 'sensitised' sheep red cells are added. If the cells lyse, free complement is present; if the cells fail to lyse, the complement has been bound by the antigen-antibody complex and the test serum may be considered to have been positive for syphilis antibodies.

(d) *Opsonisation*. This activity of antibody is especially relevant to bacteria and will



be only briefly mentioned here. Although leucocytes readily ingest inanimate foreign particles, they are frequently unable to ingest certain bacteria on account of the repellent activity of surface antigens. Antibody sensitises the bacteria so that they are phagocytosed by the leucocytes. The presence of complement is essential for opsonisation but its role is not understood.

*Hypersensitivity.* In addition to immunity, an antigen-antibody reaction may give rise to a peculiar condition known as *hypersensitivity*. This is essentially a heightened response towards invasion by foreign materials. For example, when a guinea pig is injected with some foreign protein (such as ovalbumin) after 7–10 days it develops a condition in which it is said to be 'sensitised'. If a further large challenging dose of ovalbumin is then given into the bloodstream, the animal will exhibit a general systemic shock (termed an *anaphylactic shock*) and probably die. By restricting the antigen to a particular area of the skin, *local hypersensitivity reactions* may be produced. These are termed skin or intradermal reactions and may be *immediate*, that is, they occur in 15–30 minutes and then disappear, or *delayed*, that is, they only develop after 24–28 hours.

This reaction may be used in testing for a number of animal parasite infections, especially those of helminths such as *Echinococcus granulosus* (p. 389). In practice, the test is carried out by injecting, intradermally, fluid or tissue extract of parasite or alternatively placing desiccated powered parasite on an area of previously scarified skin. In sensitised hosts, there is an immediate reaction resulting in a wheal which may fade within an hour; a delayed reaction, consisting of an area of erythema and induration, at the site of application, may also develop later.

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## CHAPTER XXXIII

# THE HOST: IMMUNITY TO SPECIFIC PARASITIC ORGANISMS

Although a great deal is known regarding development of acquired immunity to microbiological infections, much less is known about the process controlling immunity to infections of protozoans, helminths or arthropods. Interest in immunity to metazoan parasites is comparatively recent, most of the work being carried out within the last thirty years. The field has been reviewed, in full or in part, by Culbertson (1941), Taliaferro (1929), Cameron (1935), Skikhobalova (1950) and Soulsby (1957a), and has been the subject of a symposium (Chandler *et al.*, 1958). The general basis of acquired immunity to helminths appears to be the same as that with bacteria, viruses or non-living materials, that is, it is a result of specific antibody formation. There is increasing evidence, however, that whereas in bacterial infections the antigens are derived from the body substance of organisms, in metazoan infections, probably the most important antigens are *metabolic* products released by the parasite. Antibodies produced as a result of these antigens in some instances may operate by reacting with and so inactivating essential enzymes or enzyme systems. This view was first put forward by Chandler (1935) as a result of work carried out with *Nippostrongylus muris* (p. 392). After a single infection with this nematode and increasingly with repeated infections, the host was found to build up a resistance which manifested itself in inhibition of growth, development and reproduction of the worm. When *Nippostrongylus* is placed in immune rat serum, precipitations appear in its intestine and, moreover, further precipitations are given by the excretions of the worm. These results would appear to confirm the existence of metabolic antigens. Similar phenomena have been observed with other nematodes (*Ancylostoma caninum*, *Trichinella spiralis*) as well as with schistosome cercariae.

Attempts to develop practical techniques of vaccination of domestic animals against helminth parasites have, until recently, been singularly unsuccessful. Techniques

involving the use of larvae partially inactivated by irradiation with either X-rays or gamma rays (from cobalt 60) have been more successful. Development of such techniques may prove useful in the future control of parasitic helminths.

In the following sections, no attempt has been made to give a complete review of immunity to animal parasites, but rather to deal with selected interesting examples from each of the major groups.

### 33.1 Protozoa

#### 33.11 Haemoflagellates

*Trypanosoma lewisi*. This species exhibits one of the classical cases of acquired immunity and one of the earliest established for protozoans. The life cycle has already been described (p. 54). Typically, in a new infection, the trypanosome multiplies in the peripheral blood of rats for 5–6 days, after which time retardation followed by complete cessation of reproduction occurs. The organisms agglutinate in rosettes and a 'crisis' occurs on or about the 10th day when the majority are destroyed. A second 'crisis' occurs about the 35th day characterised by complete disappearance of all organisms from the blood stream. It was formerly thought possible that three antibodies might be involved in the development of immunity, (a) a reproduction-inhibiting antibody, termed *ablastin*, (b) an agglutinating antibody, and (c) a trypanolytic antibody.

All the effects can, however, be explained on the assumption that only one antibody, *ablastin*, is produced (Thillet and Chandler, 1957; Chandler, 1958). According to this view, reproduction is inhibited by *ablastin*, and the organisms become sticky and agglutinate. The agglutinated organisms are filtered out by the reticulo-endothelial system, especially by the liver and spleen, with the result that the majority suddenly disappear. This accounts for the first crisis. As the agglutinated organisms disappear, it becomes increasingly difficult for those remaining non-agglutinated to meet, and the complete removal of all trypanosomes is accomplished by normal phagocytic mechanisms. This process takes some time. There is strong evidence that *ablastin* is directed against metabolic products, probably enzymes (for convenience termed *ablastinogens*), since Thillet and Chandler (1957) have shown that serum in which trypanosomes have been cultured can induce complete immunity to non-immune rats. *Ablastin* is not absorbed out of immune serum by the trypanosomes, but is, however, absorbed by their metabolic products.

*Ablastin* has also been demonstrated in *Trypanosoma duttoni* in mice but the two *ablastins* are not quite identical. They are sufficiently near to operate in heterologous hosts. Anti-*lewisi* rat serum is effective in inhibiting *T. duttoni* in mice but anti-*duttoni* mouse serum needs to be used in large doses to be effective against *T. lewisi*.

*Leishmania*. Immunity has not been thoroughly investigated for this group of organisms. The cutaneous forms (p. 62) induce a marked immunity after a single infection. Resistance is also developed in human visceral cases but the balance between the organisms and the defence mechanisms is nearly equal. If the defence mechanism gains the upper hand, complete immunity and elimination of the parasites occur; if the invaders gain the upper hand, death results.

Laboratory animals exhibit a whole range of resistance to *Leishmania donovani* (Stauber, 1958). The hamster is completely susceptible and succumbs to introduction of a single organism. The rabbit and rat exhibit complete innate resistance and can dispose of several million parasites. The gebril and guinea pig are susceptible but soon acquire immunity.

### 33.12 Entamoebae

*Entamoeba histolytica*. There is no evidence of acquired immunity to amoebiasis in man (Hoare, 1958; Beaver *et al.*, 1956) and amoebic infections may be very persistent and last for years. Some resistance appears to develop in dogs. There is some evidence of an immunological reaction by the rat from serological tests. When amoebae are restricted to the gut (as in 'symptomless carriers', p. 31) antibody formation is not stimulated and no complement-fixation response is detected; the amoebic antigen is clearly weak and not sufficient to stimulate appreciable antibody formation. It is possible that the low-grade antigenicity of protozoans in general is due to the predominance of lipoid haptens in contrast to the carbohydrate haptens of bacteria which have powerful anti-genetic properties.

### 33.13 Haemosporidia

It is difficult to obtain an overall picture of immune responses to malarial organisms on account of the large number of species and hosts concerned. Main interest, of course, has centred on species affecting man. Each species appears to be a complex of immunologically different strains, and anti-parasite immunity is usually strain-specific.

*Host restriction*. The majority of plasmodia are host-specific although many can persist in other hosts for short periods and a few can infect a range of hosts.

In man, there are differences between the races as regards susceptibility. Negroes, for example, are generally more resistant to *Plasmodium vivax* than white persons (Russell, 1952).

Thus, in a study of semi-resistant West Africans, Miller (1958) found that in the sample studied, adults showed parasites on an average of 21 per cent of the time as compared with 85 per cent for children. Moreover, the level of parasitemia was much greater in children than in the adults. Average daily parasite counts were  $56/\text{mm}^3$  for adults and  $2,230/\text{mm}^3$  for children. The level required to induce clinical attacks in adults and children also showed differences. In adults, on occasion, counts as low as 30 were able to initiate clinical symptoms, but in children counts below 11,000 did not result in the onset of clinical disease.

*Acquired immunity.* There appear to be two kinds of mechanism involved in the development of general resistance to malaria: (a) an *antiparasitic resistance*, which inhibits survival and multiplication of the parasite and (b) an *antitoxic resistance* (= *tolerance* of some authors), due to the detoxification of the products of parasite metabolism responsible for the malarial paroxysm. According to Blackburn (1948), antitoxic resistance is not strain-specific and is only slowly developed; infants and children may thus suffer severely. Indeed, it requires almost a continuous infection over a period of fifteen years or more before a useful tolerance can be established. In contrast, antiparasitic resistance is strain-specific.

Comparatively little is known regarding the nature of antibody formation in human malarial infections. Antibody production has perhaps been most thoroughly studied in monkey malaria although it is known to occur in human cases. In monkeys, specific agglutinins, complement-fixing antibodies, and protective antibodies have been described. Within 15–45 days, agglutinins appear and in repeatedly infected animals the titre may reach 1:1,000. The complement-fixing antibody appears after the third week. The properties of protective antibody have not been precisely determined. In bird malarias, agglutinins have been demonstrated.

With some rare exceptions, no mammals can be infected by any species of bird malaria although the majority of species can infect a wide range of bird hosts. At least eight species of birds are susceptible to *P. cathemerium* and *P. relictum*, but many species reported as distinct may prove to be identical with others. The physiological behaviour of a species may also vary markedly with the host. For example, *P. cathemerium* has a 24-hour erythrocytic schizogony cycle in the canary, but a 48-hour cycle in the chick. The number of merozoites may likewise vary in different hosts.

In all acquired immunities to plasmodia, the reticulo-endothelial system plays a major role by directly phagocytosing the organisms and by elaborating antibodies which opsonise them preparatory to phagocytosis. The enlargement of the reticulo-endothelial system is characteristic of the disease and the spleen may increase to several times its normal size.

### 33.14 Coccidia

*Host restriction.* Species of coccideans have exceptionally narrow host spectra, rarely extending beyond two or three species, and usually limited to a single species. Thus,

*Eimeria stiedae* will develop in rabbits but no other mammal; *E. tenella* develops in the chicken but in no other bird. In contrast, *Isopora felis* will infect both dogs and cats.

*Acquired immunity.* Any acquired immunity is species-specific. The degree of immunity developed varies with the species, developing more rapidly and more completely in those which penetrate deeply into the tissues. Thus, a single infection of the penetrating *E. tenella* in chick is sufficient to provide complete protection against reinfection, whereas repeated infections of less penetrating species are necessary to provide full immunity.

Severe infections of coccidiosis, although inducing immunity, may ultimately result in death. Drugs such as quinoxaline and nicarbazin (Cuckler and Malanga, 1956) have been particularly effective in suppressing oocyst formation without interfering with the development of immunity (Fig. 24).

A complement-fixing antibody, with a life of about 50 days, has been detected in artificially immunised animals. Neither serum antibody nor the reticulo-endothelial system appears to play a very significant part in the development of immunity. Immunity to coccidiosis appears to be a local response related to the epithelial cells of the bile ducts or the intestine. These cells either prevent sporozoites entering or kill those which do succeed in penetrating. The mechanism of this local action is unknown.

### 33.2 Helminths

#### 33.21 General Considerations

Aspects of the immunity to helminth infections have been reviewed by Taliaferro (1940), Culbertson (1941), Skikhobalova (1950), Soulsby (1957a) and Chandler *et al.* (1958). Available evidence suggests that the various immunological mechanisms operative against parasitic helminths are fundamentally identical with the humoral and cellular mechanisms developed against protozoans, bacteria and micro-organisms. Although this view is now widely accepted, the concept of immunity to helminth infections is a comparatively recent one, dating basically from studies by Stoll (1928) on the sheep nematode *Haemonchus contortus* (p. 331), which induces partial immunity to reinfection. Stoll called this phenomenon 'self-cure', and it is clear that although he himself had the idea of immunity comparable to that developed against micro-organisms, the majority of parasitologists at that time had not seriously considered this possibility.

Stoll's actual experiment was carried out on two lambs. One was infected with 45 *Haemonchus* larvae, and the other left uninfected. On the 19th day the infected lamb passed eggs so that the pastures were now contaminated. The originally uninfected lamb began to pass eggs on the 54th day. About 10 weeks after their first egg passing, the lambs reached high peaks of egg-production of 10,600 and 13,000 eggs per gram respectively. Then there was a rapid fall to negative egg counts within 2-3 weeks. There-



after they remained negative although up to 14,000 infective larvae were ingested per day. No more were acquired and those already present were for the most part expelled, constituting the 'self-cure'.

Most early immunological studies were limited to tests of possible diagnostic value, especially for helminths of medical or economic importance such as *Echinococcus granulosus*. It is now appreciated that helminths offer a number of advantages over protozoans, bacteria or other micro-organisms for basic investigations in immunology. These advantages are fourfold (Taliaferro, 1940):

(a) they do not multiply in the body, in contrast to bacteria or protozoans, so that precise control of an effective dose is possible;

(b) they tend to mature in a specific site within the body, or, if they undergo a tissue migration, the route is well defined;

(c) they are relatively enormous in size, compared with a single bacterium or protozoan, so that individual tissues can be isolated and antigens prepared from them;

(d) immunological effects, such as the formation of an envelope around a cercaria in immune serum, can sometimes be directly observed and followed under the microscope.

Although complete immunity against helminths may be developed, an immune response is usually evidenced by stunting, inhibition of reproduction and loss of worms. *Metabolic antigens*. As already stressed (p. 380), there is increasing evidence that many of the phenomena appearing in superinfections, such as stunting, retardation of development and inhibition of reproduction, are a result of the action of anti-metabolic antibodies. These inhibit metabolism and interfere with the normal digestive and assimilatory processes of the parasite, and may be anti-enzymatic in action. The existence of this type of antibody has clearly been demonstrated for *Trypanosoma lewisi* (p. 381). The stimulating antigens in the case of helminths are believed to be the secretions and excretions of the adult or larval worms. The experimental evidence for this view has been mainly indirect and based on the formation of precipitates in the intestine or around the body openings of worms incubated in homologous antiserum. Recently, direct evidence has been produced by the demonstration that excretions and secretions collected from a helminth and injected into a host have given partial protection against a challenging infection of the same parasite. The nature of these antigenic secretions has been determined only in the case of *Nippostrongylus muris* (Thorson, 1953), where released lipases serve as antigens. Direct evidence for the existence of metabolic antigens has also been produced for *Ancylostoma caninum* (Thorson, 1956), *Trichinella spiralis* (Chipman, 1957; Campbell, 1955) and *Schistosoma mansoni* (Kagan, 1958).

If the antigenic secretion is a particular digestive enzyme, the antibody will only

be directed against this particular enzyme. Nevertheless, the precipitation taking place within the intestine will not only inhibit the enzyme itself but is likely also to effectively reduce the surface for absorption and thus further interfere with the nutritive processes. Thus an anti-enzymatic type of antibody may have both a primary and secondary effect.

The realisation that the metabolic products of helminths can serve as antigens has given new importance to the elaboration of techniques for the *in vitro* cultivation of helminths. This question is discussed further in Chapter XXXIV.

*Irradiated larvae as antigens.* A number of attempts have been made to utilise extracts of worms as antigens, but although these invoke the production of antibodies, the degree of immunity developed is always of a low order. Immunity develops much more strongly in the presence of living whole worms, presumably because of the production of metabolic antigens. Means were therefore sought to treat worms in such a way that they would be attenuated and yet still retain their ability to invoke somatic and metabolic antibodies. Most of the work has been carried out on larval nematodes as many of these undergo extensive migration in the definitive host, and although sexual maturation is usually prevented by irradiation, the migratory phase (during which the immune reaction is evoked) is comparatively little affected.

When treated with X-rays or radiation from cobalt 60, many species of larval nematodes became sterilised and yet retained their ability to induce immunity. This has resulted in the development of vaccines which can produce some degree of immunity against several nematodes of economic importance (p. 393). Whether this method will be capable of application on a scale sufficient to be of real value remains to be seen, but preliminary results have been promising.

Immunity to some of the commoner helminths is considered below.

### 33.22 Trematodes

*Fasciola hepatica.* Although complement-fixation antibodies have been detected in the serum of sheep and other hosts of *F. hepatica*, there is no evidence that effective immunity to this trematode is ever developed. In cattle infested with *F. hepatica*, hypersensitivity can be readily demonstrated and diagnosis is some 90 per cent accurate (Soulsby, 1954).

The rabbit is an excellent experimental host for this parasite. Urquhart *et al.* (1954) described a procedure for preparing an antigen from *F. hepatica*. Precipitin tests showed that the sera of infected rabbits contained antibodies which reacted with the precipitating antigen. Immunisation of rabbits prior to infection produced inhibition of development but did not reduce their numbers significantly. This probably also represents the immunological picture in other hosts of *Fasciola*.

*Schistosomes*. The immunology of schistosomiasis has been the subject of a number of investigations and knowledge in this field has been reviewed by Newsome (1956) and Kagan (1958). Most of the studies have been carried out on *Schistosoma mansoni* (p. 185) maintained in laboratory hosts such as mice and hamsters, and the rodent species *Schistosomatum douthitti* (p. 194) in the white mouse.

#### *Host restriction*

(a) *Schistosoma mansoni*. This species will develop in a number of mammals in addition to man. These include four species of monkeys (Tables 22, 25), albino mice, cotton rats and hamsters, these last making the most satisfactory laboratory hosts. Albino rats and guinea pigs possess a high natural resistance, which results in the destruction of eggs in the tissues and worms in the blood vessels. In rats, extensive infections with mature worms cannot be produced even with heavy infections of cercariae. Average infections can be produced in guinea pigs, but only by the use of massive doses of cercariae.

(b) *Schistosomatum douthitti*. The natural hosts of this species in the United States are the muskrat, the deer mouse and the meadow mouse, but in the laboratory, hamsters and albino mice are the most susceptible hosts. In rats, cats and rabbits, development is poor or abnormal. In rhesus monkeys, infections may be tolerated for two to three weeks, after which time the worms are suddenly killed and the infections rapidly terminated (Kagan, 1958).

#### *Acquired immunity to schistosomes*

*Serological immunity*. Antibodies against both adult and larval schistosomes have been detected in serum and a number of diagnostic tests based on the usual antibody manifestations have been developed. These include precipitin, flocculation, haema-glutination, complement fixation and skin tests. In addition, two unusual reactions have been developed. These are the CHR and the miracidial immobilisation test which are further considered below.

Practically every stage of the life cycle has been used for antigen—adult worms, cercariae, infected snail livers, miracidia and eggs. The method of extraction has varied considerably, saline, alcoholic or ether extracts being favoured by different workers.

Of all the tests mentioned above, perhaps the complement fixation has been the most widely used and it is still probably the most sensitive test for the diagnosis of early infections. Unfortunately, the skin test at present developed is not specific and lacks standardisation, but on account of its relative simplicity would be a diagnostic

tool of value if these difficulties could be overcome. Kagan (1958) gives an excellent review of these tests.

The *CHR test* (Cercarienhüllen Reaction of Vogel and Minnig, 1949) is of special biological interest. It is manifested by the appearance of a transparent membrane around the tail of a schistosome cercaria when placed in immune serum. This reaction is destroyed by heating the serum to 56°C. for 30 minutes. Electrophoretic studies have revealed that the CHR membrane develops only in the gamma and alpha fraction of the serum (Evans, Stirewalt and MacKenzie, 1955). The CHR reaction has been applied to both *S. mansoni* and *S. douthitti* with rather similar results. As a diagnostic tool the method has obvious limitations. It is not as sensitive as some of the other reactions and also necessitates maintaining living cercariae in the laboratory.

The *miracidial immobilisation test* is based on the immobilisation of miracidia in immune serum, and it has been found to be an exceedingly sensitive one. The method has not yet been widely used. All stages of schistosomes have been found to produce immobilisation antibodies.

*Host immunity.* Although precise experimental evidence is lacking, it is generally accepted that man and animals develop resistance to schistosomiasis. This conclusion is based on work describing a reduction in the number of cercariae developing into mature worms in subsequent infections compared with the numbers developing in the primary infection. In man, a very high degree of resistance is developed in adults over the age of thirty-five and this has been proved experimentally without question (Fisher, 1934). In mice, well documented evidence has shown that immunity towards *S. douthitti*, *S. mansoni*, *S. japonicum* and *S. spiralis* may be acquired. In all these species, there is some evidence that it is the presence of the egg which serves as the antigenic stimulus, although metabolic antigens may also be involved.

In spite of the fact that the presence of antibodies can readily be demonstrated in immune serum, it remains unproven that schistosome 'immunity' has an antigen-antibody basis. This point remains to be tested by passive transfer experiments. Many other problems related to both natural and acquired immunity need exploring. Considering the importance of schistosomes to human health, it is remarkable that so little precise information is available.

### 33.23 Cestodes

Immunity has been most strikingly demonstrated in the case of larval cestodes and much less so in the case of adult forms.

*Hydatigera taeniaeformis.* Rodents which contain few or many strobilocerci in their liver are protected against large numbers of oncospheres fed 56–105 days thereafter. Artificial immunity may be produced by repeated injections of powdered adult worms although the immunity is not as effective as that naturally acquired.

An antibody basis for this immunity has been demonstrated (Miller and Gardiner, 1934) and serum from immunised rats is effective in inhibiting cyst development in

normal rats in doses as low as 0.2 ml per 100 gram rat. Passive immunity lasts about 26-36 days.

There is evidence that the total immunity against larval *H. taeniaeformis* can be divided into an 'early' phase of acquired immunity, which prevents larvae developing to recognisable strobilocerci in the liver, and a 'late' phase of acquired immunity which results in the death of the larvae after they have formed recognisable strobilocerci.

*Echinococcus granulosus*. Most of the work with this species has been carried out on the hydatid stage. Little work has been done on the related species *E. multilocularis* (see p. 264). Complete immunity to larval infections is never developed but in subsequent infections fewer cysts become established and cysts show thicker surrounding adventitia and more calcification.

A number of serological tests for the diagnosis of hydatid cysts have been developed. These include a precipitation reaction, complement fixation, and an intradermal reaction. The latter, which is known as the Casoni Test, utilises hydatid fluid as the antigen and is the most widely used. None of these tests are absolutely reliable and false positives may occur in hosts suffering from various diseases.

*Diphyllobothrium latum*. There is no evidence for acquired immunity in animals or man infected with *D. latum* or any other pseudophyllid, although this field has been little investigated. No satisfactory serological test for the diagnosis of this organism has been developed.

*Hymenolepis* spp. The species commonly encountered in laboratory rodents are *Hymenolepis diminuta* (p. 254) and *H. nana* (p. 256) each of which shows different behaviour patterns after repeated infections.

*Hymenolepis diminuta*. This species exhibits 'premunity', that is, protection against reinfection as a result of an existing infection (in contrast to 'immunity' which is related to a *previous* one), a phenomenon common amongst cestode species. It has been shown (Chandler, 1939) that the size of worms in a secondary infection is inversely proportionate to the number of primary worms harboured. When a primary infection is eliminated by an operation, no difference was found in the rate of growth of the worms in the secondary infection. This clearly indicates that 'premunity' in cestode infections is not due to the action of an immune mechanism but to a crowding effect which probably has mainly a nutritional basis.

*Hymenolepis nana*. In the case of this species, infections can be produced either with or without a parenteral phase and strikingly different immunological responses are produced depending on the mode of infection.

If eggs are fed directly to a mouse, the cysticercoids develop in the villi before



becoming established as adult worms in the intestine. Close contact with the tissues and blood stream is thus made and true immunity develops. However, if cysticercoids grown in beetles are fed, there is no parenteral phase and no immunity develops.

### 33.24 Nematodes

*Trichinella spiralis*. It has been clearly demonstrated in a number of hosts (rats, guinea pigs and pigs) that animals which recover from an infection of *T. spiralis* resist infection. This resistance is never complete, but is transmitted in many cases from immune mothers to their young. *Trichinella* antigen appears within 24 hours in serum and precipitation has been reported to occur when worms are placed in the serum as early as the fifth day. There seems little doubt that acquired resistance is directed against the *intestinal* phase of the worm, for if infective larvae are fed to immune animals, they are eliminated without development. Inhibition is probably largely the effect of the serum antibody, but how larvae make contact with this antibody is uncertain. Undoubtedly, the worms become exposed to antibody on penetrating the intestinal wall, but it is possible that antibody may actually diffuse from the blood into the intestinal lumen.

Immunity to re-infection by *T. spiralis* has also been induced by feeding rats with larvae irradiated with X-rays or gamma rays from cobalt 60 in doses sufficient to produce sexual sterilisation, but not sufficient to prevent growth to adult size (Levine and Evans, 1942; Gould *et al.*, 1955).

The doses used were: 3,250–3,750 r. of X-rays, or 10,000 r. of cobalt 60. Excessive doses of the latter, such as 18,000 r., prevent them from developing to adults, and larvae so treated will not induce immunity.

Feeding of irradiated larvae does not induce complete immunity, but the number of adults maturing in the intestine, and the number of larvae which reach the muscles, can be measured in tens rather than in thousands (Table 44).

The excretions and secretions of *Trichinella spiralis* collected by incubating infective larvae in a nutrient fluid for two to five days can also act as antigenic materials (Chipman, 1957; Campbell, 1955). When such material is centrifuged (to remove any worm tissues possibly present) and injected daily into mice, it produces an immunity as revealed by the presence of significantly smaller burdens of adults and larvae, an effect more marked with the larvae than the adults.

*Ascaris lumbricoides*. This well-known species occurs in man, monkeys and pigs. In each of the hosts, the adult occurs as a strain specific for that host. There is, however, practically no known mammalian host specificity for the early migratory phase of *Ascaris*, and this only becomes evident when it re-enters the intestine. In the smaller mammals such as mice, rats, guinea pigs and rabbits, the *suum* strain of *Ascaris* passes



out of the body shortly after returning to the intestine. Antibodies have been detected in the sera of these hosts. In the larger mammals such as goats, sheep or cattle, it can grow to half size, and sometimes produce eggs. In man, the *suum* strain will likewise hatch but never reaches maturity.

TABLE 44

EFFECTS OF RE-INFECTION RATS, FORTY DAYS AFTER  
AN INITIAL INFECTION OF *TRICHINELLA SPIRALIS*

using: A, untreated larvae; B, larvae irradiated with 10,000 r. from  
Co<sup>60</sup> (data from Gould *et al.*, 1955)

Days after re-infection	Group A (No Co <sup>60</sup> )		Group B (Treated Co <sup>60</sup> )	
	Adult trichinae in intestine	Larvae recovered from muscles	Adult trichinae in intestine	Larvae recovered from muscles
2	17	181,000	1,912	15
	165	32,200		
4	0	166,000	848	11
	8	25,800		
6	0	164,000	42	0
	4	47,240		
8	52	101,000	9	0
	0	640,000		
10	0	156,000	0	22
15	0	190,000	0	0
	0	—		
20	0	96,000	0	496

In pigs, and probably also in man, some degree of resistance is developed, and antibodies have been detected in pig serum also. Schwartz (1959) showed that about four weeks after an initial infection, substantial numbers of larvae began to be eliminated in the faeces. This natural elimination of larvae may be associated with the final moult. It may also be related to the resistance developed as a result of stimulation during the early migratory phase.

A substantial degree of immunity may be developed by using embryonated *Ascaris* eggs as a vaccine. Soulsby (1957b) injected, subcutaneously, infected embryonated eggs on two occasions at intervals of 20 days. A control group was given 20,000 infective ova orally at the same time interval. 20 days later both groups were challenged with 100,000 infective ova, given orally. Representatives from each group were killed daily, and the number of larvae in the liver and lungs counted and the sizes measured.

In the immunised group, the number of larvae on the eighth day was small, and many were covered with precipitates and showed evidence of marked degeneration. These results are summarised in Fig. 158.

*Nippostrongylus muris*. Development of immunity to this rodent nematode has probably been more studied than any other species.

*Acquired resistance.* In *Nippostrongylus muris*, acquired immunity manifests itself by (a) stunting of growth, (b) retardation or complete inhibition of development, (c) inhibition of reproduction, (d) elimination of previously established worms, and (e) refractoriness to infection. This acquired immunity manifests itself in about 18–22 days after an initial infection of about 1,000 larvae. There is clear evidence that immunisation must

be considered in two phases (a) parenteral (stimulated by the tissue migration of the larvae), and (b) intestinal (stimulated by older larvae or adults in the intestine).

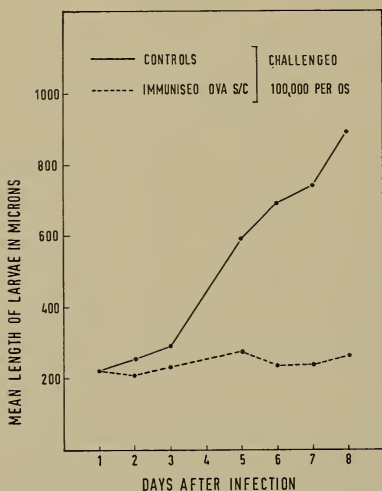


FIG. 158. Mean lengths of larvae derived from a challenge dose of 100,000 *Ascaris lumbricoides* ova per os in a control group of guinea pigs and in a group immunised by subcutaneous injection of embryonated *A. lumbricoides* ova (adapted from Soulsby, 1957a).

Demonstration of intestinal immunity has also been achieved. The parenteral phase was eliminated by feeding rats via a duodenal tube with three-day-old fourth-stage larvae, and after these had become established they were challenged subcutaneously by *Nippostrongylus* larvae. The result was almost the same as that for an infection preceded by the normal parenteral phase, but the immunity developed was not quite so strong.

When *Nippostrongylus* larvae are placed in antiserum, precipitates form throughout the intestine and at the mouth, anus and excretory pore. Thorson (1953) has shown that serum or saline in which larvae had been cultured for 24 hours contain antigens which, when injected into rats, produce antibodies which react with larvae *in vitro* to form precipitates. Injection of saline or serum, containing excretions

or secretions of larvae, into rats also produced partial protection from further infections. The secretions and excretions were further shown to possess lipolytic activity and this activity was inhibited by immune serum. Thus lipases at least can act as antigens and probably other enzymes also. Antibodies to such enzymes would interfere with the nutrition of the worms, and if lipolytic enzymes are functional in penetration, might also affect its migrational activities.

*Haemonchus contortus.* The occurrence of 'self-cure' in infections of *H. contortus* have

already been referred to as one of the earliest examples of immunity to helminths (p. 384). It has been found that an effective vaccine can be prepared by using third-stage larvae partly inactivated by radiation doses of 40,000–60,000 roentgens (Table 45), and that development of the adult worms is unnecessary for the development of immunity. When irradiated larvae are fed orally to sheep, they are still able to undergo a limited migration; this period of activity is sufficient to stimulate the production of a

TABLE 45

IMMUNITY OF LAMBS AGAINST *HAEMONCHUS CONTORTUS*

Groups of 5–7 lambs were infected with larvae irradiated with various doses as indicated. They were challenged with 8,000 normal infective larvae 117 days later, and autopsied after a further 4–6 days (data from Jarrett *et al.*, 1959a)

Group	Roentgen dose	Mean no. of worms on challenge
1 (B)	10,000	5
2 (B)	20,000	0
3 (B)	40,000	200
4 (B)	60,000	0
5 (B)	100,000	442
7	Double infection	620
8	Control	2,042

high degree of immunity to reinfection (Jarrett *et al.*, 1959a). Serological evidence suggests that antigens are produced at the third ecdysis and it has been speculated that the antigen initiating the self-cure reaction is the exsheathing fluid believed to be produced at this ecdysis (Soulsby *et al.*, 1959). It is clear that work in this field is progressing rapidly and that the detection and isolation of purified and specific metabolic antigens cannot be long delayed.

*Dictyocaulus viviparus*. The pattern of immunology is somewhat similar to that of *H. contortus*. Third-stage larvae partially inactivated by exposure to 40,000 roentgens form a suitable vaccine, and calves doubly vaccinated with this vaccine are almost completely resistant to infection when challenged with 10,000 normal larvae (Jarrett *et al.*, 1959b). The clinical effects of this vaccination are mild and the method is likely to be of economic value in preventing parasitic bronchitis in calves.

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## CHAPTER XXXIV

# IN VITRO CULTIVATION OF ENDOPARASITES: PROTOZOA

### 34.1 General Account

It is generally recognised that many aspects of the biology of parasitic organisms can only be satisfactorily studied when they are grown in culture free from other organisms. This is especially true of physiological and biochemical studies, and, in common with the pattern found with free-living organisms, advances in knowledge in these aspects of parasitism have run closely parallel with the development of suitable culture methods.

The discovery that metabolic products excreted or secreted by parasites can act as antigens, has lent a new urgency to the need to develop efficient methods of *in vitro* cultivation. Only by so doing will it be possible to investigate, and perhaps isolate, the metabolic antigens, an end which, apart from its purely biological interest, is of considerable economic importance.

Cultivation of an organism in the absence of other organisms is termed 'axenic' (Greek *a* = free from; *xenos* = a stranger), a term introduced into biological literature by Baker and Ferguson (1942) and now in widespread use. A culture in which one other species of organism is present is called 'monoxenic'; and, if many are present, 'polyxenic'. The term 'axenic' is replacing older, less precise terms such as 'aseptic' or 'sterile', although the time-honoured term '*in vitro*' is worth retaining, as it has come to be associated with a special type of culture involving a liquid or solid medium in a tube or flask-type container. Culture terminology has been discussed by Dougherty (1953) and that now in general use is summarised in Table 46. The problems associated with the axenic culture of invertebrate metazoans were reviewed in a recent symposium (Dougherty *et al.*, 1959).

Techniques for the axenic cultivation of parasitic micro-organisms such as bacteria, yeasts and fungi have been established for many years, and are in widespread routine use in diagnostic laboratories.



Many methods for protozoans are related to or adapted from those used for bacteria or other micro-organisms, and like those, offer many variants or improvements of the original techniques. Culture techniques for protozoans in general have been easier to develop than those for parasitic helminths, in which very special problems are involved; these are reviewed in detail in a later chapter (p. 409). It is not intended

TABLE 46  
TERMINOLOGY FOR GROWTH OF ORGANISMS, MOSTLY UNDER  
KNOWN (GNOTOBIOTIC) CONDITIONS  
(from Dougherty *et al.*, 1959)

<i>Terms</i>	<i>Number of associated organisms</i>
Gnotobiotic . . . .	None, or known species only
Axenic . . . .	None
Synxenic . . . .	One, or more, known species
Monoxenic . . . .	One known species
Dixenic . . . .	Two known species
Trixenic . . . .	Three known species
Polyxenic . . . .	Several, to many, known species
Agnotobiotic, or xenic . . . .	Unknown

here to review all the methods used to culture parasitic protozoans or to give practical details of the preparation of media. The main object of this section is to outline the principles behind the various culture methods and deal with a small number of selected examples.

### 34.2 Intestinal Protozoa

*General.* Physico-chemical conditions in the vertebrate intestine are complex, and it might be expected that *in vitro* culture of intestinal forms would prove to be difficult. In practice, however, by using monoxenic or polyxenic cultures, it has been found possible to establish relatively stable conditions; these have been sufficiently close to those occurring in the gut to permit growth and multiplication of numerous species of intestinal protozoans.

A variety of media have been developed. The original method of Boeck and Drbohlav (1925) made use of a culture tube as shown in Fig. 159. The medium is diphasic, the solid phase being in the form of an ordinary bacteriological slant covered to a variable extent by the liquid phase. One of the most reliable media is Dobell's 'HSre' medium. In 'HSre' medium, the solid phase is composed of heat-coagulated protein (i.e. horse serum, inspissated at 80° C.), and the liquid phase of egg-albumen

diluted with isotonic saline. A loopful of sterile rice starch is added as a source of carbohydrate. Details of the preparation of these media are given in standard manuals (e.g. Hoare, 1949; Simmons and Gentzkow, 1955).

When a culture of an intestinal protozoan (e.g. *Entamoeba histolytica*) is initiated by inoculating a tube with a loopful of faeces containing cysts, or better still with a loopful from another culture, the organisms sink to the bottom and grow mainly in the interface region between the two phases where the starch has settled (Fig. 159). The cultures commence by being aerobic, but become rapidly anaerobic due to the usage of available oxygen by the aerobic bacteria. A drop of methylene blue, for example, is rapidly bleached. In a flourishing culture, a complex microflora of aerobic and anaerobic bacteria occurs and more or less 'balanced' environmental conditions prevail for a time. Acid or alkali produced by concomitant bacteria is rapidly neutralised within wide limits by the system itself, so that other adjustments are unnecessary. The presence of bacteria is essential for the growth of many species of *Entamoebae*; their role in the nutrition of the amoebae is further considered below.

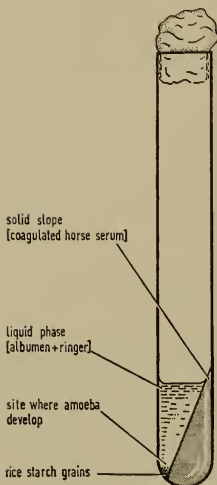


FIG. 159. Tube with Boeck-Drbohlav diphasic medium for *in vitro* culture of intestinal entamoebae, and certain other intestinal protozoans (after Dobell, 1942).

The Boeck-Drbohlav system, or modifications of it, have been most successful when used with protozoans of the caecum and large intestine such as species of *Entamoeba*, *Trichomonas* and *Balantidium*, whose main diet consists of bacteria and intestinal debris. Intestinal protozoans, such as *Giardia lamblia*, whose natural habitat is the duodenum (i.e. a region of high nutritional level, p. 16), cannot be successfully cultured by this method.

Although diphasic media are still widely used, a number of satisfactory monophasic media have also been developed. *In vitro* techniques for the most commonly cultured species are considered below.

### 34.21 *Entamoeba histolytica*

This species grows readily in the Boeck-Drbohlav media or modifications of it. A number of monophasic media have also been used, one of the most satisfactory being that developed by Shaffer and Frye (1948) and known as the S-F medium. This

medium makes use of an unidentified streptobacillus (believed to belong to the genus *Bacteroides*) in a thioglycollate medium.

S-F medium is prepared as follows. The streptobacillus is incubated for 24 hrs. at 37°C. in fluid thioglycollate medium (Baltimore Biological Laboratory No. 136C) containing 1 per cent glucose and 1 per cent rice starch. After incubation, this medium is centrifuged horizontally at 2,000 r.p.m. for 45 mins. The supernatant fluid, known to contain 30–70 million viable bacterial cells per ml, is collected and used as a base for the medium. 2.5 ml of this fluid are added to culture tubes containing 0.1 ml horse serum and 0.7 ml saline (0.85 per cent NaCl) which contains 1,000 units penicillin G. potassium. The medium is then inoculated with *E. histolytica* and layered with petrolatum to maintain anaerobic conditions. In this medium, amoebae propagate very actively and have characteristic growth curves depending on the strain of *E. histolytica* used.

For a great many years, the role played by bacteria has been under investigation. There are a number of possibilities:

(a) The actively multiplying cells or some part of them may be ingested and serve as food.

(b) Some metabolic product or products of bacterial multiplication may be released into the medium and act as growth factors.

(c) The bacteria may produce some set of conditions such as optimal pH or O<sub>2</sub> reduction.

(d) More than one of these possibilities may be operative.

It has been brilliantly demonstrated by Shaffer, Schuber and Key (1958) that in S-F medium the penicillin present induced the bacteria to give off bubble-like 'round bodies' (Fig. 160) which were then ingested by the trophozoites of *E. histolytica*. In this



FIG. 160. Effect of penicillin on bacteria in the Shaffer-Frye medium for the cultivation of *Entamoeba histolytica*. A. an untreated bacterium; B. bacterium giving off 'bubble-like' bodies under the influence of penicillin. Diagrammatic only.

medium, then, the actual ingestion of the substance of the bacteria appears to be essential for active growth and reproduction of the amoebae. That this view is correct is strikingly substantiated by the fact that streptococcus variants which do not produce the round bodies do not support *E. histolytica*.

The role of other species of bacteria in other culture media is uncertain but it may be related to their size or their ability to produce similar bodies under certain conditions. Ingestion of particulate material certainly seems essential to the amoebae, and Reeves *et al.* (1957) have shown that the streptococcus of the S-F medium can be replaced by a particulate factor present in embryo extract.

### 34.22 Other Parasitic Amoebae

Many other common parasitic amoebae such as *E. coli*, *E. gingivalis* and *E. muris* have been cultured by methods similar to those used for *E. histolytica*. The temperature range is important for amoebae other than those from warm-blooded hosts. The reptilian species *E. invadens* (p. 35), for example, has an optimum range of cultivation of 24–30° C. (McConnachie, 1955). This species can also be cultured axenically by the substitution of liver slices for the bacteria used in normal cultures (Lamy, 1948).

### 34.23 Intestinal Flagellates

The Boeck-Drbohlav system or modifications of it are also suitable for the cultivation of many species of intestinal flagellates, and all the intestinal flagellates of man, except *Giardia lamblia*, and many species from animals have been cultured *in vitro*. Diamond (1954) made a study of the ability of twenty-eight different culture media to support *T. gallinae*, and found the most satisfactory were Ringer-Loeffler serum, saline-Loeffler serum and saline-serum. *Trichomonas muris* from the mouse (Fig. 9) is a convenient organism for preliminary culture work. Axenic culture of a number of

TABLE 47

#### COMPOSITION OF JOHNSON AND TRUSSELL'S DIPHASIC MEDIUM FOR AXENIC CULTURE OF *TRICHOMONAS VAGINALIS*

For details of preparation see Simmons and Gentzkow (1955)

Solid Phase	bacto peptone . . . . .	32.0 gm
	bacto agar . . . . .	1.6 gm
	cysteine hydrochloride . . . . .	2.4 gm
	maltose . . . . .	1.6 gm
	bacto-liver infusion . . . . .	320.0 ml
	Ringer . . . . .	960.0 ml
0.5 per cent methylene blue . . . . .		0.7 ml

Liquid Phase—human serum 12 ml

species has been achieved. Table 47 details the medium employed by Johnson and Trussell for the axenic culture of *T. vaginalis*.

### 34.24 Intestinal Ciliates

These have proved rather more refractory to *in vitro* cultivation than intestinal flagellates. In general, modifications of the Boeck-Drbohlav system have been used for blood-temperature ciliates such as *Balantidium*. In a diphasic medium of coagulated horse serum overlaid with diluted serum plus rich starch, the organisms conjugate

regularly at 37° C. but at 25° C. conjugation ceases. Amphibian ciliates such as *Opalina* and *Nyctotherus* may be cultured by similar methods at room temperatures. The axenic culture of *Opalina* has been reported by Yang and Bamberger (1953) in a diphaseic egg/serum-saline medium.

### 34.3 Blood and Tissue Protozoa

#### 34.31 General Account

In contrast to intestinal forms, protozoans from blood or tissue habitats can only be cultured under axenic conditions. Infective material, whether blood or tissue, must thus initially be obtained in a sterile condition. Blood and tissue-dwelling protozoans lack a cytopharynx, so that all nutrient must be absorbed through the body surface, and in general the conditions occurring in the normal blood or tissue sites are more difficult to reproduce. The methods developed have been most successful in the case of the haemoflagellates (leishmanias and trypanosomes) and less so with the sporozoans.

#### 34.32 Haemoflagellates

*Routine methods.* These flagellates were first cultured in the condensation liquid of a solid blood-agar slope and this method, or modifications of it, is still used.

The medium most widely employed is blood agar, more commonly known as NNN medium (after Novy, MacNeal and Nicolle). It consists of 14 gm agar and 6 gm NaCl dissolved in 900 ml water. This is dispersed in tubes and one third volume of sterile defibrinated rabbit's blood added to the agar cooled to 45–50°C. Slants are prepared without butts and the organisms are inoculated into the water of condensation formed on the surface.

This method works well for the cultivation of *Leishmania* spp., *Trypanosoma cruzi* and in non-pathogenic flagellates of the *lewisi* group. It is not successful for the trypanosomes of man such as *T. gambiense* and *T. rhodesiense*.

In cultures of haemoflagellates, it is especially interesting to note that the stages which develop in *in vitro* culture are those corresponding to the stages occurring in the insect-vectors, so that the nutritional requirements and/or the environmental conditions for metamorphosis and differentiation of the later stages are not being satisfied. The optimum temperature range is 20–25° C. which is approximately the temperature at which the arthropod vectors normally live. A number of attempts have been made to develop defined media for haemoflagellates, so far without success. Citri and Grossowicz (1955) have produced a medium which is 'nearly' chemically defined; its composition is shown in Table 48.

*Cultivation in chick embryos.* A number of species such as *T. rhodesiense*, *T. brucei*, *T.*

TABLE 48

COMPOSITION OF THE PARTIALLY DEFINED MEDIUM FOR THE CULTIVATION OF *TRYPANOSOMA CRUZI*

(from Citri and Grossowicz, 1955)

Basal medium		Growth factors replacing tomato juice in TJ medium			
	g/l		μg/ml		μg/ml
NaCl . . . . .	4.0	<i>p</i> -Aminobenzoic acid . . . . .	0.1	Riboflavin* . . . . .	1.0
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O . . . . .	3.0	Biotin . . . . .	0.2	Thiamine* . . . . .	1.0
KH <sub>2</sub> PO <sub>4</sub> . . . . .	0.5	Choline . . . . .	3.0	Cobalamin . . . . .	0.0001
Casein hydrolysate (enzymic)† . . . . .	10.0	Folic acid . . . . .	2.5	Ribonucleic acid . . . . .	80
Crystalline serum albumin* . . . . .	2.0	Inositol . . . . .	150.0	Cytidylic acid . . . . .	20
Glucose* . . . . .	2.0	Nicotinamide . . . . .	15.0	Creatine . . . . .	20
	mg/l	Pyridoxin . . . . .	2.0	Creatinine . . . . .	20
Haematin* . . . . .	10.0	Pyridoxal . . . . .	2.0		
Tween 80 . . . . .	10.0	Pyridoxamine . . . . .	2.0		

\* Seitz-sterilised and added aseptically to the autoclaved medium; final concentration as indicated above.

† A product of Bios Laboratories Inc., purified by adsorption on charcoal at pH 4.5.

*equiperdum* and *T. evansi* have been cultured successfully in developing chick embryos. This is a well-known technique now used routinely for virus culture.

The method consists essentially of cutting a window in a hen's egg of ten-day embryonation, and injecting approximately 0.5 ml of inoculum containing the trypanosomes into the allantoic cavity. The chick blood becomes heavily infected; the embryo usually dies within four or five days.

*Cultivation in tissue cultures.* Tissue cultures have also been used for the cultivation of a number of haemoflagellates which have tissue stages. *T. cruzi* is readily cultivated in embryonic heart muscle or spleen-cell culture. Within the host cells, the trypanosomes multiply by repeated binary fission in the leishmanial stage, the resulting individuals assume the trypanosome form and escape into the surrounding media. Later, they penetrate into proliferating host cells, where they again become rounded and the cycle is resumed.

### 34.33 Malarial Parasites

*In vitro* cultivation of haemosporideans has proved to be particularly difficult, due to a number of problems discussed in detail later (p. 407).

Early workers did not attempt to culture the tissue stages, as these were not known



before 1940. It was then realised that such stages could be grown in cultures of susceptible tissue using standard tissue-culture procedures. In practice, these methods have proved efficacious and the exoerythrocytic stages of some avian forms have been maintained *in vitro* for over a year.

*In vitro* cultivation of erythrocytic forms has proved to be more refractory. This is not surprising since the experimental hosts used were either birds or monkeys, and reliable information on the physical and chemical properties of the blood of these animals was required before much progress could be made. Most of the fundamental work on the cultivation of erythrocytic forms has been carried out on the simian species *P. knowlesi* (p. 82), although another simian species *P. cynomolgi* (p. 82) and the avian species *P. lophurae*, as well as three species of human malaria, *P. vivax*, *P. falciparum* and *P. malariae*, have also been used. *P. knowlesi* has the advantage that quantities of parasites can be produced for *in vitro* work, the host red cells are non-nucleated, the asexual cycle is short (24 hours), and the organism is highly pathogenic; it will also produce clinical malaria in man.

#### *Culture of exoerythrocytic stages in tissue culture*

The extensive literature in this field is reviewed by Pipkin and Jensen (1958). One method has been to utilise materials from infected animals as the tissue-culture source. This is an effective method, since cells and parasites are planted simultaneously in the culture flasks. The second method is to cultivate cells from uninfected animals and then to infect them with parasites. This latter method has been singularly unsuccessful, due to contamination when the sporozoites were introduced or to the inability of the sporozoites to penetrate, or for other reasons.

The most usual technique has been to use fragments of tissue embedded in plasma clots. These produce cultures too thick for microscopic examination, and examination must be carried out on stained material. For microscopic study under phase contrast, hanging drop cultures and monolayer cultures have been used with success. By cultivating explants alternatively in hanging drops and plasma clots, and by using roller tubes, parasites have been maintained in culture for as long as a year. The procedure below is that used for *P. gallinaceum* (Lewert, 1950).

The culture medium consists of a clot of chick plasma/Tyrode (1:1) with just enough chick-embryo extract to permit clotting of the plasma, covered with a supernatant liquid of 5 parts chick serum and Tyrode (1:1) and 1 part chick-embryo extract. The usual tissue-culture procedures using roller tubes are followed, and the supernatant fluid requires changing twice weekly. Tissue is transferred to a hanging drop in a Carrel flask and when a new culture is required, a piece of fresh tissue (brain, liver or spleen) is placed beside the infected fragment in the hanging drop. The newly infected tissue is then transferred to a roller tube for another 10-14 days. This method provides a relatively simple method of maintaining

EE forms, transference to hanging drops and the addition of fresh tissue when necessary being all that is required to maintain cells. A natural infection may be recommenced at any time by injecting infected tissue into chicks. In this type of culture, only parasites which actually invade tissue cells are capable of survival and multiplication; those free in the medium rapidly degenerate.

If prolonged observation of exoerythrocytic stages is required, the parasites must be continuously nourished for normal development to take place. If the culture medium is unchanged, a gradual accumulation of metabolites will create unfavourable conditions. A number of types of perfusion chambers have been developed, the use of which enables the culture fluid to be renewed at intervals. These techniques are reviewed by Pipkin and Jensen (1958).

### *Culture of erythrocytic stages*

It is clear that such stages could be cultured in two ways, either intracellularly, while still within the erythrocyte, or extracellularly, after being freed from the erythrocyte. The first approach has been the one most generally attempted, although some experiments have been carried out with the parasites freed from their host erythrocytes.

Whichever approach is adopted, the basic problems to be solved are:

- (a) provision of a physical environment with a pH, oxygen tension, CO<sub>2</sub> tension, osmotic pressure, etc., simulating that of blood.
- (b) provision of suitable nutrients for the growth and development of the parasites.
- (c) provision of culture conditions which permit the elimination of waste metabolites.

*Culture of intracellular parasites.* Since the life of a mammalian red cell has been estimated to be approximately 120 days, it should be *theoretically* possible to maintain the organisms for a period approaching this. In practice, however, even the most successful methods only maintain the organisms for about seven days.

Early experiments showed that the rapid conversion of glucose to lactate took place at a rate of about 25–75 times that of the normal red cells themselves, and this produced deleterious changes to both normal and parasitised cells. It was clear that any method developed must provide for the rapid elimination of lactate and other waste products of metabolism. Two types of culture techniques were devised at Harvard by Geiman *et al.* (1946) using slightly different principles, and these were the rocker dilution technique and the rocker perfusion technique. Geiman (1951) has given a useful summary of the development and modification of these methods.

*The rocker-dilution technique.* The method consists essentially of a closed vessel containing parasitised blood with nutrient fluid in the ratio of 3:1, through which a gas phase of 5 per cent CO<sub>2</sub> and 95 per cent O<sub>2</sub> is circulated.

The vessel used is the 'boat' shown in Fig. 161 with a stoppered central opening and two narrow lateral ports through which the gas phase circulates. During incubation at 38–39°C. the 'boats' are rocked on a rocker at the rate of about 20 times per minute, so that the red cells are kept in suspension. The

original nutrient fluid contained some 28 constituents, including inorganic salts, organic compounds, vitamins, amino-acids, and para-aminobenzoic acid but a simpler fluid was later developed (Table 49).

TABLE 49

COMPOSITION OF SIMPLIFIED CULTURE MEDIUM FOR *PLASMODIA*

Inorganic	Gm/l	Organic	Gm/l
MgCl <sub>2</sub> .6H <sub>2</sub> O . . . .	0.203	Glucose . . . . .	3.00
CaCl <sub>2</sub> . . . . .	0.056	Glycerol . . . . .	0.25
NaCl . . . . .	5.825	Na Acetate 3H <sub>2</sub> O . . . .	0.25
KCl . . . . .	0.410		
Na <sub>2</sub> HPO <sub>4</sub> . . . . .	0.242		
Na <sub>2</sub> CO <sub>3</sub> <sup>a</sup> . . . . .	1.480		
KH <sub>2</sub> PO <sub>4</sub> . . . . .	0.057		
		Added separately	
		Liver extract <sup>b</sup> . . . . .	10 ml/l
		Amino acids <sup>c</sup> . . . . .	80-160 mg/l
		Ascorbic acid . . . . .	3 mg/l

<sup>a</sup> After equilibration with CO<sub>2</sub>—pH. 7.45 ± 0.10. Freezing point 0.60 ± 0.02° C.

<sup>b</sup> Concentrated solution, Lederle, 3649-34.

<sup>c</sup> Parenamine, 15 per cent solution, enriched with glycine and histidine.

As the rocker-dilution technique is essentially a closed system, this method is particularly suitable for biochemical studies and it has been of some value in testing drugs for anti-malarial action. The daily increase of parasites with the method is about

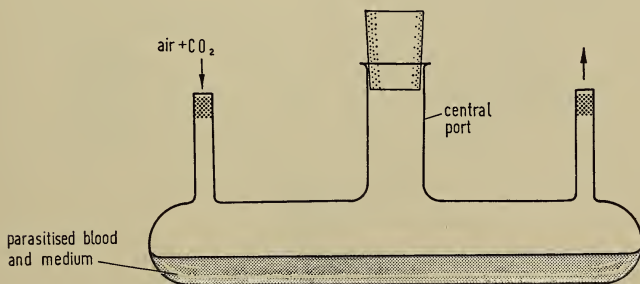


FIG. 161. 'Boat' used for the rocker dilution technique (after Geiman, 1949).

3-4 fold which approaches the *in vivo* rate and is high enough to enable sub-cultures to be made.

*The rocker-perfusion technique.* This method is designed to maintain the physiological conditions in the parasitised blood for a longer time. It consists essentially of whole heparinised blood or defibrinated blood containing the parasites in a cellophane tube

surrounded by nutrient medium. The cellophane provides for an interchange of permeable nutrients from the medium and dialysable crystalloids from the blood. Several variations of the basic principle have been used, the one which is shown in Fig. 162 uses about 10–15 ml of parasitised blood.

The culture vessels are made up of a 250 ml beaker with the flared edge removed and with a side arm attached; into the latter is fitted a rubber vaccine port. The port serves for sterile introduction and withdrawal of blood with a needle and hypodermic syringe. Four holes on top serve for the inflow and outflow of nutrient medium and for the maintenance of the  $\text{CO}_2/\text{O}_2$  gas phase as used in the rocker dilution technique described above. The medium enters the culture vessel through one of the glass tubes

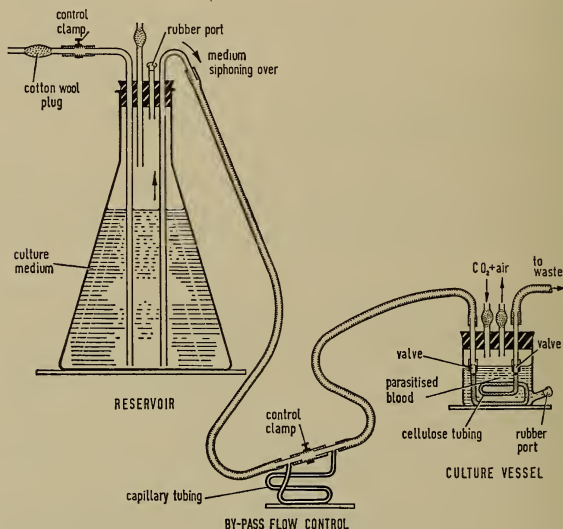


FIG. 162. Perfusion apparatus for the *in vitro* cultivation of *Plasmodium knowlesi* (after Geiman *et al.*, 1946).

in the bung and passes through cellophane tubing (threaded over a solid coiled glass rod) tied firmly to the inlet and outlet tubes. The nutrient medium is contained in a reservoir consisting of a 2-litre Erlenmeyer flask and its flow into the culture vessel is controlled by the by-pass capillary coil which, when accurately adjusted, permits a flow of about 1500 ml medium in 24 hrs.

As in the rocker dilution technique, the culture vessel is rocked gently, during incubation. The blood in the culture vessel continually bathes the cellophane coils and

draws its nutrients through the cellophane membrane, at the same time eliminating crystalloids or waste products of metabolism by dialysis.

The success of these elegant techniques may be judged by the fact that although the average multiplication obtained over a large range of experiments is about three- to four-fold, multiplication up to 9-11 fold has been obtained. Provided cultures are sub-cultured every 24 hours, they may be maintained for periods of up to a week and the terminal cultures of the series are still capable of producing acute malaria in experimental animals. *Extracellular culture of erythrocytic stages.* From the point of view of obtaining basic information concerning the metabolism of the malarial parasites, intracellular cultivation is unsatisfactory because it is not possible to determine whether a particular constituent of the medium is being utilised by the parasite or the blood cell.

Extracellular culture, however, presents very special difficulties, one of the initial ones being to free the parasites from their host cells. This can be achieved by the following method.

Duckling red cells heavily infected with *Plasmodium lophurae* are exposed to the action of guinea-pig complement and a haemolytic antiserum prepared in rabbits. This results in a suspension containing clumps of agglutinated, haemolysed red cells, free red-cell nuclei and free parasites. Most of the parasites retained in haemolysed but unbroken erythrocytes become free during the first day of incubation. Parasites may be separated by centrifugation, the freed parasites remaining in the supernatant fluid (Trager, 1955).

Trager (1955) has reviewed some of the problems of extracellular cultivation. He has developed a complex medium in which it has proved possible to obtain a complete erythrocytic cycle and the beginning of a second cycle. In the best experiments, 90-95 per cent of the parasites retained their normal appearance after three days *in vitro*, and in some experiments the majority persisted for four days. Even under the most favourable conditions, however, degeneration set in on the fifth day.

Significant success has also been achieved with the mosquito phase of the avian parasite *P. relictum* (p. 82). Using a complex defined medium plus 70 per cent chicken serum and 2.5 per cent chick-embryo extract at pH 6.8 and 20-22° C., Ball and Chao (1960) were able to culture the mosquito phase of *P. relictum*. These were initially recovered from the stomach of the vector (*Culex tarsalis*) and cultured in Carrell flasks, small Petri dishes or hanging drop slides. In the best results obtained, the oocysts continued to increase in size for five and, more rarely, eight days and underwent a threefold increase in diameter. The rate *in vitro* was generally less than that *in vivo*. In the older oocysts (started in culture before sporozoites could be seen), development proceeded until sporozoites could be detected within the cysts.



Results such as these are encouraging, but it is clear that extensive further investigations will be necessary before it will be possible to carry out the entire life cycle of *Plasmodium* extracellularly.

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## CHAPTER XXXV

# IN VITRO CULTIVATION OF ENDOPARASITES: HELMINTHS

### 35.1 General Account

The cultivation of parasitic helminths presents a number of problems, some of which are common to those of protozoan cultivation discussed in the previous chapter. The very size of the majority of helminths, however, as well as the complex nature of their life cycles, presents special problems which do not arise in the cultivation of micro-organisms. These problems will be considered in detail below.

Whereas even ten years ago, the concept of culturing a parasitic worm through all its developmental stages, from egg to adult, outside the host, would have been considered an idealistic one, today, due to the development of new technical methods, this goal has been nearly reached in the case of several helminth species. The major difficulties to be overcome in achieving this end may briefly be summarised as follows:

(a) Parasitic helminths live in biological habitats, such as the intestine, blood stream, lymph channels, viscera, etc., whose physico-chemical characteristics are often imperfectly known and whose properties may be difficult to reproduce *in vitro*.

(b) Many live in non-sterile habitats, so that treatment by antiseptic or antibiotic procedures may be necessary before axenic culture can be attempted. This difficulty can be overcome in the case of a number of helminths by using larval stages from sterile habitats, instead of adults, for the initial stages of cultivation.

(c) All species feed on biological materials (e.g. bile, blood, mucus, tissue exudates, intestinal contents, etc.) which are complex in nature and origin, and whose nutritional properties are difficult to replace by artificial media.

(d) The nature of the natural biological environment is such that rapid diffusion of metabolic waste materials from the parasite is usually possible and accumulation of toxic materials seldom occurs. Any *in vitro* method developed must similarly provide culture conditions suitable for permitting the removal of waste materials.

(e) Many parasites are of considerable size. This problem presents acute technical difficulties in the case of cestodes, which may reach many yards in length.

(f) Many helminths have complex life cycles involving the development of larval stages in one or more intermediate hosts. Each stage in development may thus require different physico-chemical conditions and may possess different nutritional requirements.

(g) The life cycles of very few parasitic helminths are known in complete detail. Although the broader aspects of the life cycles may be known, histological, cytological, cytochemical and biochemical pictures of the entire processes of maturation and development are not available. Without such detailed pictures, it is difficult to establish satisfactory criteria, by means of which development *in vitro* can be assessed. This is especially so in the case of cestodes and trematodes.

The relative magnitude of any one of these problems varies enormously between groups and between individual species. Many nematodes, for example, have free-living stages in their life cycles, whereas trematodes and cestodes, except for a brief free-living period in some cases, are exclusively parasitic. Although still exceedingly difficult, the problems of cultivating a parasitic nematode from egg to adult are less formidable than in the case of cestodes and trematodes. Also, the transparent nature of many nematodes makes it possible to decide whether or not growth and morphological differentiation are proceeding, under any particular set of culture conditions. With cestodes and trematodes which, for the most part, are opaque, recourse must be made to special cytological or histochemical methods to adjudge development. Progress in the cultivation of cestodes or trematodes has been greatly retarded pending the development of techniques to achieve this end.

The initial establishment of axenic conditions was formerly a major problem in this field, but the advent of antibiotics has provided a powerful tool for the elimination of contaminating micro-organisms, and this problem may now be considered to be one of relative insignificance.

In the section below no attempt has been made to survey this whole field, but merely to give several examples from each group to illustrate the basic problems involved.

Axenic culture of invertebrate metazoans, including the parasitic groups, has been the subject of a symposium, the published account of which adequately summarises much of the field (Dougherty *et al.*, 1959a).

## 35.2 Trematodes

### 35.21 Adult Trematodes

*Fasciola* and *Schistosoma* have been the most widely used experimental organisms. These species possess some food reserves (mainly glycogen), with the result that when placed in isotonic medium (saline or serum) and incubated at body temperature, some endogenous energy sources are available and worms may 'survive' for considerable periods. In most early experiments, survival was adjudged by movement. It is now recognised that survival *per se* as a criterion is of little value in culture work of this nature unless it is considered together with the normal physiological condition of the organism. The establishment of precise criteria, indeed, may be considered to be one of the first pre-requisites to culture attempts. In the case of helminths, prolonged 'survival' often indicates *abnormal* conditions, for the metabolic rate may be so depressed by the cultural conditions that prolonged periods of survival become possible.

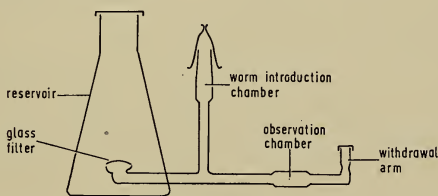


FIG. 163. Vessel used by Robinson for the maintenance *in vitro* of *Schistosoma mansoni* (adapted from Robinson, 1956).

#### *Schistosoma mansoni*

In its natural habitat, the portal system, *S. mansoni* (p. 185) ingests and metabolises quantities of blood, the female taking in more than the male, presumably on account of the greater synthetic demands of egg-production. The portal blood system is rich in glucose and amino acids, and it can be assumed that any culture media capable of sustaining growth in *S. mansoni* must contain these basic constituents. This conclusion is borne out by the results of Clegg (1959) referred to below.

Schistosomes will, however, survive for long periods of time in serum provided that glucose is added as an energy source, that the medium is renewed frequently, and that axenic conditions are maintained. Robinson (1956) devised an apparatus for maintaining *S. mansoni* in serum and glucose which has some advantages over the conventional culture tube in that the medium can be readily renewed from a reservoir (Fig. 163) and

worms may be easily observed during cultivation. In the best results with this apparatus, copulation and egg production were observed to take place in adult worms and 'survival' for several weeks resulted. Although survival was good in the serum-glucose medium, there was no evidence that 'normal' metabolism was being maintained or that viable eggs or sperms were produced. Senft (1958) has devised a complex apparatus in which the culture fluid can be circulated, but no results are available.

Although the theoretical aim is to culture a cercaria to an adult schistosome, several workers used schistosomulae from mice as a starting point in their culture attempts. Thus Cheever and Weller (1958) used schistosomulae recovered from mice on the 16th-18th day after the intraperitoneal injection of cercaria. In a number of tissue-culture media supplemented with blood cells or other substances, considerable growth, as adjudged by size, was obtained. In mixture 199 + human serum, for example, a size increase of 600 per cent was reported but the larvae never grew to full size or reached sexual maturity. Histochemical or metabolic studies were not carried out, so that apart from growth in size there was no evidence that the rate of development *in vitro* paralleled that normally occurring *in vivo*.

Clegg (1959), with schistosomulae of a similar age from mice, found that by using a medium of 50 per cent inactivated rabbit serum, 50 per cent Hank's saline and 1-2 per cent homologous rabbit red blood cells enriched with 0.25 per cent lactalbumin hydrolysate, (medium renewed every four days), growth *in vitro* up to a stage where testes and sperm developed could be obtained. Growth up to 4 mm occurred in 28 days as compared with 50 days of the earlier workers. This worker adopted the critical approach of comparing, by means of cytological and histochemical criteria, development *in vitro* with optimal development *in vivo*. The criteria used were essentially those of Bell and Smyth (1958), considered in detail later, and the use of this approach enables results to be expressed graphically (Fig. 164). More recently, Clegg (personal communication) has succeeded in growing a schistosomula to the egg-producing stage *in vitro*, but full details of these results are not yet available. These results constitute a substantial breakthrough in this field.

### *Fasciola*

This organism has been a favourite one for culture attempts, as it is usually locally obtainable. Many of the so-called attempts to 'culture' *Fasciola* have been little more than maintenance at a survival level. Provided asepsis is maintained, it will 'survive' for considerable periods in serum or isotonic saline at body temperature, often holding on to the walls of the vessel by means of its suckers. Clegg has shown

that under such conditions, however, development becomes abnormal within a few hours, as adjudged by the histological and cytological condition of the organs, and especially of the testes.

In *Fasciola*, and trematodes in general, one of the difficulties is to provide a culture vessel with physical conditions comparable to those of the host, so that (a) attachment, (b) feeding, and (c) elimination of waste materials can take place. Cellulose tubing as used for cestodes (Fig. 166) provides a useful artificial 'gut'; its surface is pliable and

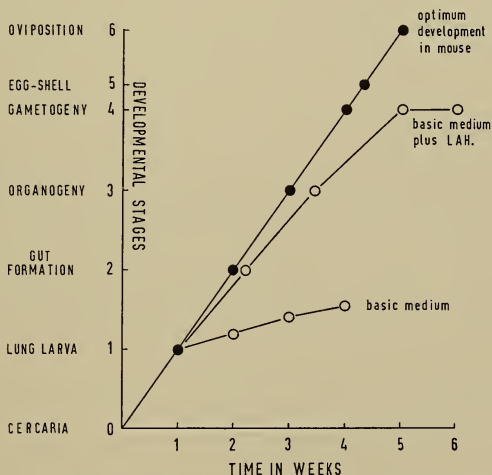


FIG. 164. Development of *S. mansoni* *in vitro*, compared with development *in vivo* (after Clegg, 1959).

suitable for attachment, it is semi-permeable, thus permitting elimination of waste materials, and it has the added advantage of being transparent. The best results so far obtained with this technique by Clegg, using Hedon-Fleig's saline and fresh specimens from experimentally infected rabbits, have had an average period of 'survival' of 18 days, with a maximum of 34 days.

#### Miscellaneous trematodes

On account of their lower metabolic demands, trematodes from poikilothermic hosts will survive in physiological saline at room temperature for several days, even

under non-sterile conditions. Under axenic conditions, survival for very long periods, often running into months, is possible. Thus, *Haplometra cylindrica*, aseptically removed from a frog, remains alive in Hedon-Fleig's saline for periods of 48-105 days (Dawes and Muller, 1957). The presence or lack of glucose in the medium in the case of this organism has no effect on the longevity, although in the absence of glucose endogenous glycogen reserves drop from 1.5 per cent. to 0.8 per cent (wet weight). In all cultured specimens of *Haplometra*, the rate of meiotic division shows a sharp decline from about 24 divisions per 1,000 spermatocytes *in vivo* to 14 per 1,000 in worms cultured in dextrose. As with *Fasciola* and cestodes (p. 421), the histological condition of the testes provides a useful criterion for assessing the general physiological condition of the organism.

### 35.22 Larval Trematodes

No serious attempts have been made to cultivate the larval stages of trematodes other than cercariae or metacercariae. The sporocyst and redia stages occur in sterile sites in the molluscan host, and theoretically should lend themselves to axenic culture attempts.

Cercaria or metacercaria are essentially juvenile flukes, and a number of workers have attempted to grow these to sexually mature adult flukes *in vitro*. In discussing attempts of this nature, it is convenient for purposes of reference to divide the process of maturation into a number of phases (see also p. 209).

(a) Cell multiplication: the period during which a larva increases its number of cells without these cells undergoing differentiation.

(b) Body shaping: a stage corresponding to segmentation in cestodes, but often not clearly defined in trematodes; the body takes on its general adult features.

(c) Organogeny: the outlines of the main genitalia, especially the tubular uterus, begin to appear.

(d) Early gametogeny: the appearance of the primordia of the testes and ovaries.

(e) Late gametogeny: the appearance of active gametes.

(f) Shell formation: the vitelline cells reach maturity and pass into the uterus preparatory to releasing egg-shell material.

(g) Oviposition: fully formed, shelled eggs appear in the uterus.

#### *Progenetic metacercaria*

Many trematode metacercariae reach an advanced state of morphological development in the intermediate host, whereas others remain relatively undifferentiated. At one



end of the scale are metacercariae such as those of *Fasciola hepatica* and *Diplostomum phoxini*, which are poorly differentiated and undergo all their sexual maturation stages in the definitive host. At the other end are the progenetic metacercariae of species such as *Coitocaecum anaspidis* which reach full sexual maturation in the intermediate crustacean host (p. 151).

In between these two extremes are species whose metacercariae reach varying degrees of differentiation. It is clearly not as difficult to bring about the *in vitro* maturation of advanced progenetic larvae, which may already contain well-differentiated anlagen of both male and female genitalia, as to bring about the maturation of undifferentiated larvae such as those of *Fasciola* or *Diplostomum* which are required to undergo considerable tissue growth before reaching maturation.

Although progenetic metacercariae are parasites of many common animals, few serious attempts have been made to bring them to maturity *in vitro*.

Experiments with the strigeid *Posthodiplostomum minimum* (Ferguson, 1940) illustrate the type of result so far achieved. Metacercariae of this species occur encysted in the viscera of the pumpkinseed sunfish. They can be readily removed and excysted by pepsin treatment and sterilised by repeated passage through sterile salines. The natural definitive host of this species is a heron but maturation will also take place in chicks. The anlagen of all the major genitalia are present in the metacercariae, and these are in a sufficiently well advanced state for maturation to take place within 36 hours in the chick gut.

It is not known whether these larvae will become mature in Tyrode solution alone on incubation at the definitive host temperature. In the medium of Tyrode (5:3 dilution) chicken serum and yeast extract, Ferguson (1940) obtained maturation of metacercariae within four days and eggs appeared in the culture tubes. This maturation time corresponds to nearly twice that required *in vivo*. The eggs produced in these cultures were not morphologically normal, nor were they viable. The artificially matured adults were likewise morphologically abnormal in that the vitellaria were poorly developed compared with adults matured *in vivo*. In these experiments, then, the medium used is clearly not sufficient to satisfy the requirements of the last stages of maturation. The possible reasons for this are discussed in the next section.

#### *Undifferentiated metacercariae (e.g. Diplostomum phoxini)*

Development of an undifferentiated metacercaria to an adult trematode involves considerable tissue synthesis and is concerned with more fundamental problems than attempts to mature a metacercaria already in the organogeny stage of development.

The metacercariae of *Diplostomum phoxini* occur in the brain of the minnow (p. 204) from which they are readily removed in a sterile condition.

As stressed earlier, it has become increasingly evident in culture work of this nature that it is not sufficient to merely know the broad outlines of the life cycle of the experimental organism but to have available a detailed cytological, histological and histochemical picture of its whole process of maturation. When these are available, precise criteria for growth and development can be applied and experimental results need not be based on survival or other such inadequate criteria.

In *Diplostomum phoxini*, the criteria developed (Bell and Smyth, 1958) for the various phases (p. 209) are as follows:

Phase 1. *Cell Multiplication*. An outburst of intense mitotic activity characterises this first stage of development, as with all embryonic tissue. It is difficult to establish a growth response for this stage: increase in wet weight, total DNA or mitotic activity offer possible solutions. Estimations based on DNA content would be the most reliable but have not, so far, been applied to this kind of material. Inhibition of mitosis by colchicine followed by aceto-orcein squashes offer an approximate method which enables the growth-stimulating powers of a medium to be assessed. The limitations of such a method are generally recognised, but in the preliminary stages of culture work the technique is sufficient to distinguish between growth and mere 'survival' and to permit the screening of obviously unsuitable media.

The method, briefly, involves treatment of a larva with  $10^{-4}$  colchicine in saline for 4 hours at  $40^{\circ}\text{C}$ , preparing an aceto-orcein squash and counting the total number of inhibited mitoses seen in the squash. In the case of *Diplostomum*, this method is sufficient to demonstrate strikingly the differences between various media. In a bird gut, a metacercaria of this species shows 150-200 inhibited mitoses after 24 hrs. and this is taken as representing the standard to be arrived at *in vitro*.

Phase 2. *Body shaping*. In *Diplostomum*, after 24 hours *in vivo*, the oval body outline is replaced by the bilobed condition (Fig. 82) typical of the adult strigeid. This change in body shape is not a precise criterion, although recognisable by an experienced observer.

Phase 3. *Organogeny*. Not well defined in this species, but characterised by the appearance of the anlagen of the genitalia as seen in whole mounts or aceto-orcein squashes.

Phase 4. *Early gametogeny*. Easily recognisable in aceto-orcein squashes and characterised by the appearance of the early stages of spermatogenesis.

Phase 5. *Late gametogeny*. A very precisely defined criterion. The time taken for mature spermatozoa to appear, their quantity and activity, serve as marking a precise phase in the maturation process. Spermatozoa may be observed in living specimens pressed under a coverglass or in aceto-orcein squashes.

Phase 6. *Egg-shell formation and vitellogenesis*. As the worms mature and release their eggs, the cells of the vitellaria grow in size and commence to synthesise the egg-shell precursors (p. 143) in their cytoplasm. These precursors give strong reactions with stable diazotates, e.g. Fast Red Salt B, giving characteristic colours, often of considerable intensity. Histochemically, the presence and extent of development of the vitellaria are thus relatively easy to test for, and this method has proved invaluable in comparing the development of the vitellaria in forms cultured *in vivo* and *in vitro*.

Phase 7. *Oviposition*. Characterised by the appearance of fully formed eggs. If these are numerous they can be detected in living specimens by gently compressing under a coverglass. The colour of the egg shell, its contours or size may all serve as useful criteria, although the fertility and potential embryonation of the miracidium must serve as the ultimate criterion.

*Development in vitro*. In liquid media, such as gluco-saline enriched by serum or embryo extract, for example, survival may be for 5 or 6 days, but the highest number of mitoses recorded after 24 hours was 40 as compared to 150–200 in the bird gut in the same period (Bell and Hopkins, 1956). When semi-solids are used, much higher mitoses counts are obtained and the most satisfactory medium so far developed has been an egg-yolk/albumen/saline mixture which provides a medium of a constituency suitable for ready ingestion by the worm (Bell and Smyth, 1958).

The culture procedure used is as follows. The medium may be used in any one of a number of containers: 25 ml screw-top vials are convenient. Cultivation is carried out at 40° C. and the culture vessels are shaken briefly every 1–2 minutes by an electric shaker controlled by a simmerstat or connected to the thermostat circuit of the water bath. This simulates intestinal movement and assures the ready removal of waste products of metabolism from the vicinity of the worm. In the egg-yolk/albumen/saline medium, the mitosis rate is as high or higher than that obtained *in vivo* (Table 50). Ovaries and testes develop, and active spermatozoa, which appear in masses in the receptaculum seminis, are formed. After about 84 hours' incubation, eggs are finally formed. Eggs produced to date *in vitro* have been thin-shelled and morphologically abnormal; tests with the diazo reagent reveal that the vitellaria remain almost undeveloped.

The pattern of maturation *in vitro* with this species thus closely follows that of *Posthodiplostomum* in that normal development of the final stages of maturation has not been obtained. This suggests that abnormal synthesis of the phenol-protein precursors of the egg-shell material occurs due to nutritional deficiencies in the medium. This deficiency could be a *gross* deficiency, a shortage of protein or carbohydrate, for example, or a *micro*-deficiency of some growth factor or factors (e.g. an 'essential' amino acid, vitamin or other growth factor) forming a vital link in the synthetic processes of shell formation, or a combination of a number of these factors (Smyth and Clegg, 1959).

The processes of egg and egg-shell production are ones involving a protein synthesis of a high order and must make enormous demands on the nutrition of the

TABLE 50

CRITERIA RECOMMENDED FOR THE RECOGNITION OF DEVELOPMENTAL PHASES IN THE TREMATODE  
*DIPLOSTOMUM* AND THE GESTODE *DIPHYLOBOTHRUM*

(after Bell and Smyth, 1958)

Phase	Time in host		Criterion recommended	Method of detection
	<i>Diplostomum</i> (hr.)	<i>Diphylobothrium</i> (days)		
(1) Cell multiplication . . .	0-24	0-1	Mitoses counts	Aceto-orcein squashes after colchicine treatment
(2) Segmentation or body shaping .	12-24	1-2	Division into proglottids ( <i>Diphylobothrium</i> ) or regions ( <i>Diplostomum</i> )	Direct observation on living material or aceto-orcein squashes
(3) Organogeny . . .	24-48	2-3	Appearance of uterus and testes primordia	Squashes or whole mounts
(4) Early gametogeny . . .	36-40	4-5	Appearance of 'rosette' and 'comma' stages in spermatogenesis	Squashes
(5) Late gametogeny . . .	40-48	5-6	Appearance of mature spermatzoa	Squashes or unstained teased
(6) Egg-shell formation and vitellogenesis	55-60	6-7	Presence of egg-shell precursors in 'vitelline' cells	Histochemical tests on whole specimens. Diazo + ve, catechol + ve
(7) Oviposition . . .	60-72	7-8	Appearance of fully formed egg	Direct observations on living material or catechol-treated whole mounts

organism. The early phases of maturation, organogeny and gametogeny, are chiefly concerned with cell multiplication and reorganisation, and the nutritional demands of these phases are apparently being satisfied by the yolk/albumen/saline mixture. This is not the case in the final stages, as indicated by the abnormal egg development.

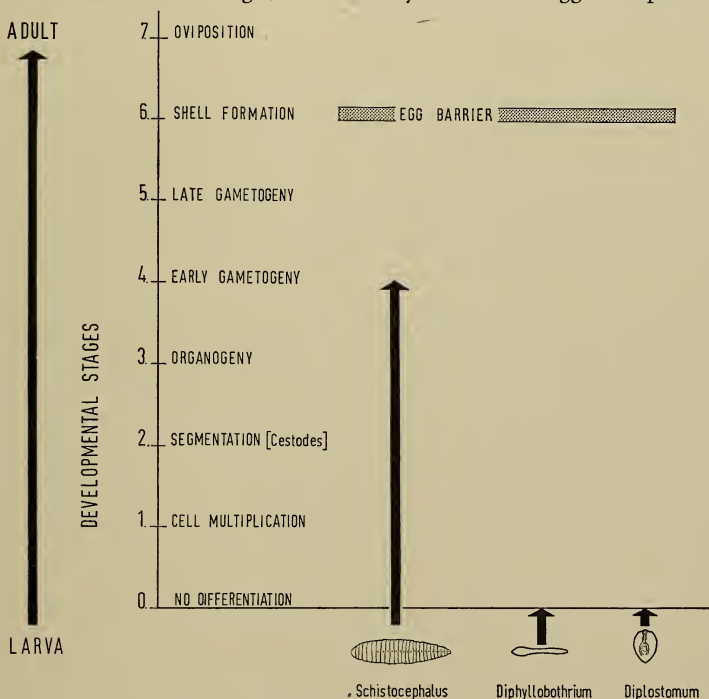


FIG. 165. Stages of maturation in cestodes and trematodes and the position of the larvae of *Schistocephalus solidus* (p. 248), *Diphyllobothrium dendriticum* (p. 239), and *Diplostomum phoxini*. The plerocercoid of *S. solidus* exhibits progenesis, and possesses the anlagen of the male and female genitalia; the remaining larvae are morphologically undifferentiated (after Smyth, 1959).

These final stages of maturation in trematodes thus constitute what has been termed an 'egg-barrier' (Fig. 165) and solution of the problems surrounding this present a challenge to future workers. It is interesting to see that in cestodes (p. 423) and parasitic

nematodes (p. 429), the definitive stages of maturation *in vitro* present comparable problems.

In trematodes, then, it is likely that *in vitro* maturation up to spermatogenesis at least could be achieved for many species, but that the later stages of maturation may present formidable problems.

### 35.3 Cestodes

*In vitro* cultivation of cestodes presents special difficulties not encountered with other groups of helminths, due mainly to the tapelike shape, the gigantic size of the strobila in some species, and the fact that they are almost exclusively intestinal parasites. The technical difficulties in providing a suitable culture vessel and sufficient medium for a species such as *Diphylobothrium latum*, the strobila of which may be 40 feet long, are clearly of an unusual order. Very special methods will be necessary to overcome such problems. In culture, small species such as *Hymenolepis diminuta*, or *Diphylobothrium dendriticum*, show a tendency to tie themselves in knots, an action which results in cytological degeneration at the site of the knotting. *In vivo*, knotting is apparently prevented by the fact that the strobila lies flattened against the gut wall.

A further major difficulty in cestode culture is the nature of the media. Cestodes show a greater degree of metabolic dependence (p. 4) on their host than trematodes, being dependent on the host to supply the digestive enzymes necessary to hydrolyse large food molecules. A suitable culture medium for cestodes must therefore contain the nutritional requirements in a molecular form suitable for ready assimilation through the cuticle (i.e. simple carbohydrates, amino-acids, and other relatively small molecules). Suitable media for cestode cultivation are thus likely to approach those used in tissue culture.

*Initial cultivation attempts.* Since cestodes are intestinal parasites, on removal from the host they are covered with a mucus film rich in micro-organisms such as yeasts, bacteria and fungi. Early workers attempted to use antiseptic washing procedures in an attempt to obtain strobila in a sterile condition. No significant success was achieved with these kinds of technique. With the advent of antibiotics, the problem of surface sterilisation becomes possible, but most workers have preferred to use larval cestodes from sterile environments as initial experimental material.

#### 35.31 Adult Cestodes

Some preliminary attempts to culture adult cestodes have been made, but these have been singularly unsuccessful. There is little to be learned from listing 'survival'



times of cestodes, as reported in the literature, since in few of these early experiments was growth reported to have taken place and precise criteria for assessing growth and development were not applied. The early literature is summarised by Smyth (1947).

Most cestodes of homoiothermic vertebrates will 'survive' for several hours in isotonic saline at host temperatures and most early experimental work was based on experiments of this nature. Cestodes of poikilothermic vertebrates, on the other hand, may survive much longer, even for several days, due to the lower metabolic rate and the comparatively high food reserves. It is generally assumed that the metabolism of cestodes, freshly removed from a host and cultured under such conditions, remains 'normal' for the first hour or so, and most routine biochemical studies on respiration and general cestode metabolism are based on this kind of material. Experiments based on cultivation periods longer than this should be accepted with caution, as striking cytological changes may occur under abnormal culture conditions, especially in nutrient-deficient media.

### 35.32 Larval Cestodes: Progenetic Pseudophyllidean Larva

The majority of pseudophyllidean plerocercoids reach only an undifferentiated stage in their most advanced larval condition (Fig. 99). However, certain species, notably *Schistocephalus solidus* (Fig. 102) and *Ligula intestinalis* (Fig. 107), have progenetic plerocercoids in which the anlagen of the genitalia develop while still within the fish host. Maturation of the former in the definitive host thus only involves differentiation from Stage 4 to 7 (Fig. 165), a process which takes place within 36 hours in the host gut. Techniques have been developed which enable this process to be achieved *in vitro*. *Schistocephalus* plerocercoids are rich in food reserves (especially glycogen which makes up more than 50 per cent of the dried weight), with the result that sufficient energy is available from endogenous sources to satisfy all the demands of maturation without provision of exogenous food materials.

The plerocercoids of *S. solidus* are found in the coelomic cavity of the stickleback *Gasterosteus aculeatus* (Fig. 105) from which they may be removed aseptically without difficulty and transferred to sterile culture medium.

Preliminary experiments established that plerocercoids rapidly develop into sexually mature adults if incubated in isotonic media such as saline or broth at 40° C. (temperature of definitive bird host). Development, when first obtained *in vitro*, was abnormal (Smyth, 1946), as evidenced by the fact that: the eggs were infertile; spermatogenesis was abnormal as indicated by presence of necrotic cells; and insemination had not occurred as spermatozoa were lacking from the receptaculum.

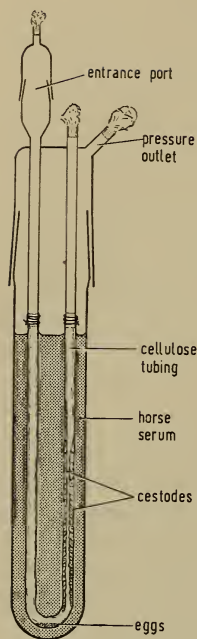


FIG. 166. A modified culture tube incorporating an artificial 'gut' of cellulose tubing (semi-permeable) used for axenic culture of *Schistocephalus solidus*. The tube is incubated at 40° C. and shaken continuously to assist the diffusion of metabolic waste products through the cellulose tubing. Compression of the strobila within the tubing is essential to assure fertilisation (after Smyth, 1959).

It later became apparent that for normal maturation to take place *in vitro* more elaborate culture procedures, more nearly paralleling the physico-chemical conditions of the gut, were required. The procedures which were ultimately successful (Smyth, 1954, 1959) involve: the use of a highly buffered medium to counteract the effect of acidic metabolic products; cultivation under semi-anaerobic (deep-tube) or anerobic conditions, to prevent premature oxidation of the phenolase-phenol-protein complexes in the shell-producing vitellaria (p. 229); compression within an artificial gut of seamless cellulose tubing, a necessary pre-requisite for insemination and fertilisation (Fig. 166); and agitation of the culture medium by vigorous lateral shaking to assist in the diffusion of waste metabolites through the cellulose tubing. Horse serum or Ringer-Locke containing  $\text{CaCO}_3$  are suitable media for this species, since all that is required here is for the medium to provide a suitably buffered physico-chemical environment in which the larva can mature using its endogenous food reserves.

The nature of the culture tube used is of importance. If the cellulose tubing is omitted and larvae cultured free within the medium, maturation proceeds normally since eggs and spermatozoa are formed, but *insemination does not take place* and sections of the mature strobila of cultured worms show that the receptaculum in each proglottid is empty. On the other hand, in sections of worms matured within cellulose tubing, the receptaculum always contains spermatozoa. For normal insemination to occur, gentle lateral compression is thus required. Presumably, the presence of a closely applied surface enables the cirrus to be bent round into the vaginal pore of the same or adjacent proglottids or into one of another worm in the same culture tube.

Eggs from larvae matured within cellulose tubing under optimal conditions show 50–88 per cent fertility and hatch out normal infective coracidia capable of continuing the *in vivo* cycle. Such eggs are unquestionably 'normal' so that the

life cycle of this cestode can now be completed without the intervention of the natural definitive host (Smyth, 1959).

If larvae are matured *in vitro*, without being enclosed within cellulose tubing, only stray spermatozoa reach the receptacula and the eggs are 95 per cent infertile. Nevertheless, these infertile eggs will frequently embryonate under stimulating conditions not understood. The resultant coracidia which develop are roughly half the size of the normal coracidia and fail to hatch on exposure to light. Such coracidia are haploid in genetic constitution, since insemination and fertilisation have not taken place.

Attempts to complete the rest of the life cycle of *Schistocephalus solidus*, that is the coracidium-proceroid-plerocercoid stages have not been successful so far. More detailed knowledge of the various habitats, the copepod haemocoel and the teleost coelom, may be required before much success in this direction is likely.

### 35.33 Larval Cestodes: undifferentiated plerocercoids

Unlike those of *Schistocephalus*, the majority of pseudophyllidian plerocercoids are undifferentiated morphologically and poor in food reserves, with the result that they must carry out substantial tissue synthesis and differentiation before sexual maturity can be attained.

To produce such an order of growth clearly presents a more fundamental problem than the mere differentiation of progenetic larvae. A species which has been studied in some detail is the avian parasite *Diphyllbothrium dendriticum* whose life cycle has been described earlier (p. 245). The organism may be conveniently grown in the albino rat, and maturation *in vivo* is extraordinarily rapid; it requires only 6-7 days, a time range which brings the organism within the range of *in vitro* culture. By examining rats fed at 24-hourly intervals, and making suitable morphological and histochemical preparations, it is possible to build up a picture of the normal development pattern with which the development *in vitro* can be compared. As in the case of trematodes, maturation can be divided into seven phases (p. 418) corresponding approximately to the degree of morphological development attained on successive days during the 7-day period of maturation in a rat. Precise criteria can be established for each stage of development (Table 50; Bell and Smyth, 1958).

In poorly nutrient media, such as serum, 'survival' may be as long as ten days, but the mitosis rate is low and virtually no development results (Fig. 167). In more nutrient medium, such as dilute embryo extract or lacto-albumen hydrolysate, mitosis rates are higher and differentiation up to Stage 2 or 3 may be attained, but the time required for such limited differentiation is 10-29 days and the larval stobila tend to tie themselves in knots with subsequent cytological degeneration.

A recent approach (Smyth, 1958, 1959) has been used to overcome this difficulty.

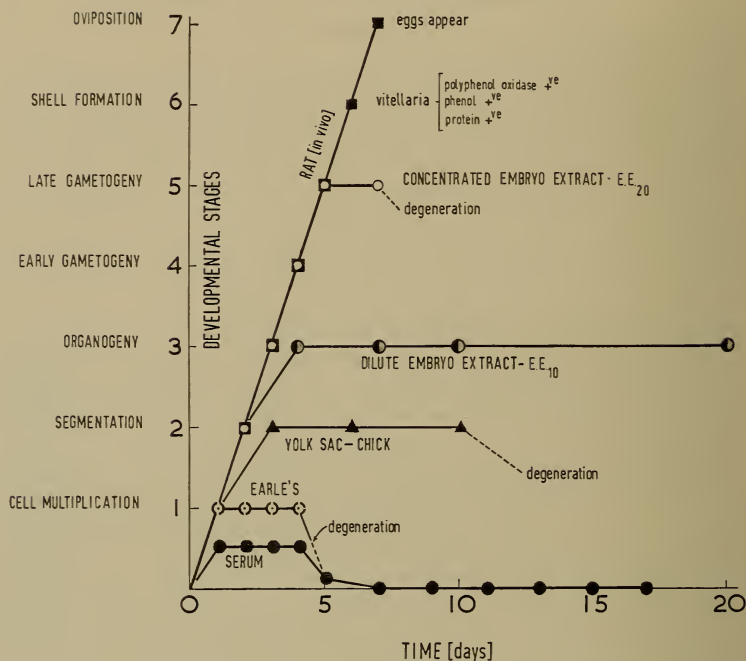


FIG. 167. Development of whole plerocercoids or plerocercoid fragments of *D. dendriticum* cultured *in vivo* or *in vitro* (after Smyth, 1959).

This consists of culturing small excised fragments of plerocercoids 1–2 mm long (Fig. 168) instead of entire larvae. In dilute duck or chick embryo extract, fragments become segmented and develop traces of genital anlagen (Stage 3). In more concentrated extract (CEE<sub>20</sub>\*), fragments become segmented by the second day, develop genital anlagen by the third day and by the sixth day become differentiated into recognisable

\* Confusion exists in the literature as to the method of expressing concentration of embryo extract. The extract is prepared in most laboratories by adding a volume of saline equal to the weight of tissue, i.e. 50 ml Earle's to 50 gm tissue. In this text, the supernatant obtained after centrifuging this macerate is 50 per cent extract = CEE<sub>50</sub>. CEE<sub>50</sub> diluted 1:1 with saline is then CEE<sub>25</sub>. Weinstein and Jones (see p. 431) refer to the supernatant as CEE<sub>100</sub>.

proglottids containing a cirrus, a sac, coiled uterus, ovary primodia and testes capsules. This degree of differentiation corresponds to stage 5 in Fig. 165. The testes capsules develop and contain spermatozoa.

A striking feature of these experiments is that although an advanced stage of differentiation is reached, no cytoplasmic growth in size takes place in the proglottids. The result is that 'miniature' proglottids are formed, each  $\frac{1}{5}$ – $\frac{1}{10}$  of the normal size of those in a rat. Beyond this point tissues undergo degeneration or autolysis.

These results resemble those obtained with trematodes (p. 420) and nematodes (p. 429) in the failure to complete the final stages of maturation. This is not unexpected,

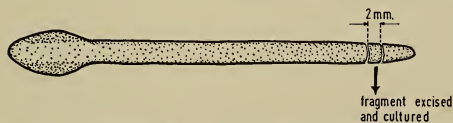


FIG. 168. Position of fragments excised from plerocercoids of *Diphyllobothrium dendriticum* for *in vitro* culture (after Smyth, 1959).

for the formation of the egg-shell material is a process involving considerable protein synthesis by the cells of the vitellaria and must make enormous demands on the organism's metabolic pool. The problem of further increasing the nutritional value of the medium to permit cytoplasmic growth and to provide for the demands of the final maturation stages remains to be solved. This is likely to be more difficult with this group than with trematodes or nematodes, on account of the absence of a gut, since only materials of the size of breakdown products of proteins, carbohydrate and fats can be utilised. Addition of various supplements (e.g. serum, lactalbumin hydrolysate) have not been beneficial to date.

The use of excised tissue, at least in the pseudophyllids, overcomes some of the difficulties inherent in normal culture procedures using entire strobila, but it remains to be seen if 'normal' maturation can ever be achieved by this method. If the nutritional problems of the final maturation stages can be overcome, the laboratory culture of relatively gigantic forms such as *D. latum* should become possible. Only further work will reveal whether this technique or modifications of it can be satisfactorily extended to other groups.

### 35.34 Larval Cestodes: cyclophyllidean larvae

Like pseudophyllidian plerocercoids, many cysticerci will survive in balanced saline containing glucose for long periods without undergoing further development.

For example, the strobilocercus of *Hydatigera taeniaeformis* has been maintained at 37° C. for 24 days in Ringer and glucose (Wilmoth, 1945). Survival is longer in saline re-inforced with biological media such as serum, ascitic fluid or embryo extract, and in some cases growth has been claimed. Thus, hydatids of *Echinococcus granulosus*, when cultured in sheep ascitic fluid plus hydatid fluid are reported growing to 20–30 times their original volume (Coutelen, 1927). Similar results were reported by the same worker with larval *Coenurus serialis* from a rabbit. Many experiments of a similar nature using other species of cysticerci have produced the same kind of result.

Much of this work has been uncritical, since little attempt was made to determine whether growth of cytologically normal tissue was obtained and whether larvae retained their infectibility for the definitive host. More critical experiments have been carried out with *Echinococcus multilocularis*, a species whose hydatids are capable of proliferating by exogenous vesicles in a number of mammalian hosts (Rausch and Jentoft, 1957). In vole embryo extract media containing serum, hydatid tissue underwent proliferation and produced scolices at a rate equivalent to that occurring in suitable mammalian hosts. Some of these larval scolices were infective since the tissue underwent proliferation when introduced (by injection) into the peritoneal cavity of a natural intermediate host. The presence of HeLa cells (a strain of cells widely used in tissue culture work) appeared to assist the development by acting as a substrate to which the larval tissue became attached; its presence also permitted the aggregations of vesicles developing from a common origin to remain intact.

It is clear from these results that, in general, more progress has been made in the cultivation of pseudophyllids than of cyclophyllids. This is largely due to the progenetic nature of the plerocercoids in several of the species used, and to the fact that more is known regarding the detailed cytology and cytochemistry of maturation in the Pseudophyllidea.

As stressed earlier, cestode cultivation presents special problems not encountered in other groups, the chief of which is to provide the nutritional requirements in a form suitable for absorption and assimilation. Much work remains to be done in this direction.

### 35.4 Nematodes

#### 35.4I General considerations

Studies on the cultivation of nematodes have been carried out on three types of material:

- (a) free-living soil nematodes (e.g. *Rhabditis* sp.)



(b) nematode parasites of invertebrates (e.g. *Neoaplectana glaseri*)

(c) nematode parasites of vertebrates (e.g. *Nippostrongylus muris*).

Several species of soil nematodes, as well as those parasitic in invertebrates, have been cultured through numerous generations under axenic conditions. Such a degree of success has never been achieved with nematode parasites of vertebrates. In this latter group the 'egg-barrier' (Fig. 165), that is the definitive stage of maturation culminating in fertile egg-production, has never been passed. Eggs so far produced *in vitro* have been infertile.

In the case of nematodes of invertebrates and vertebrates, the media used have been complex and have usually contained tissue homogenates, serum and other supplements of biological origin. In the case of soil nematodes, substantial progress has been made towards the development of chemically defined media, although a completely defined media has not been developed.

Nematodes have generally proved more responsive to *in vitro* culture attempts than the parasitic helminths for the reasons already discussed. They are not so sensitive to changes in the physico-chemical properties of the medium as are cestodes and trematodes; they appear to be well equipped with digestive enzymes; and finally, since they are transparent or semi-transparent, the growth response to various media may be readily followed visually without having recourse to microtome, cytological or histochemical methods. Indeed, the ease with which the growth and development of the various stages can be followed visually may, perhaps, have led to the neglect of other methods, which might have provided valuable clues to the deficiencies in the medium or in the cultural conditions provided.

### 35.42 Free-living Nematodes—*Caenorhabditis briggsae*

Although this is essentially a free-living, self-fertilising, hermaphroditic soil nematode with a life cycle similar to that of *Rhabditis maupasi* (p. 285), its axenic cultivation is worth discussing here since it throws light on the nutritional requirements of parasitic species in general. The available data on the culture of this species is too voluminous and detailed to record in full, but it has been surveyed by Dougherty *et al.* (1959b) and Nicholas *et al.* (1959).

In the presence of bacteria at 20° C. *C. briggsae* grows to about 5,000 times its mass. In order to compare the effect on growth and development, Dougherty *et al.* (1959b) have developed criteria which are listed in Table 51. This illustrates the use of such criteria for comparing growth responses in different media.

In purely defined media, *C. briggsae* has been grown through one generation and

a limited number of  $F_1$  produced. It has been speculated that worms are able to grow through one generation, or a little beyond, because a store of one or more dietary essentials may accumulate in the egg. Once this material is used up, growth ceases due to its absence in the diet.

It has been shown that in addition to a defined diet containing amino acids, an energy source (glucose), vitamins and growth factors, salts, and other structural compounds, an undefined factor termed Rb was essential. This factor has been found

TABLE 51  
TYPICAL RESPONSES OF *C. BRIGGSÆ* TO BASAL MEDIUM GM-16  
WITH AND WITHOUT SUPPLEMENTATION

Observations	5 per cent LPF-C plus 5 per cent ALE (5-23)		1 per cent LPF-C	None
Days to reach $\frac{1}{2}$ mature size . . . . .	1.5	6	11	—
Days to reach adult size . . . . .	5	9	—	—
Generation I time, days . . . . .	6	10	—	—
Number of F <sub>1</sub> in first 24 hours . . . . .	24	8	—	—
Generation II time, days . . . . .	5	9	—	—
Population at 20 days . . . . .	2,500	100	1	—

(Dougherty *et al.*, 1959b)

to be present in a number of biological sources such as liver protein fraction C (LPF-C) and chick embryo extract (CEE). Even in supplemented media the host growth so far realised with *C. briggsæ* in axenic culture is sub-optimal as compared with growth in the presence of bacteria. At 20° C. maturation takes four to five days in axenic culture instead of about three days at 20° C. in the presence of bacteria.

Practical details for axenic culture of this species are given by Dougherty *et al.* (1959b). Improvements in technique now permit growth studies to be carried out using only 0.2-0.3 ml media per culture tube.

### 35.43 Nematode parasites of Invertebrates—*Neoeplectana glaseri*

The species most used for *in vitro* studies have been those parasitic in the larvae or adults of soil-inhabiting beetles. One species in particular, *Neoeplectana glaseri*, has been extensively cultured, especially by Glaser and Stoll, and has now been maintained in culture for nearly fourteen years.

*N. glaseri*, although a true parasite since it attacks and develops in insects, is also a saprozoic organism, for it may eventually kill its host and live on its carcass. It is

relatively insensitive to a wide range of physico-chemical conditions and can withstand considerable changes of pH (4.5–7.5) and osmotic pressure.

The cultural techniques developed for this organism are discussed by Stoll (1959), who also reviews the previous literature.

*N. glaseri* is dioecious and ovoviviparous; it becomes infective after completing the second moult. If it does not reach a host (or culture media equivalent) it is able, as a 'dauer' larva to survive on its food materials for several months. The average time to complete a generation is 6.8 days and the average number of offspring (of mated individuals) is about 225.

In culture, it is convenient that at about 3–4 weeks (in kidney culture; see below), the infective larvae begin migrating from the culture mass to the wall of the test tube in long strands composed of many thousands of worms. Since these larvae are all dauer, their introduction into new cultures gives a base line of developmental response which can be readily followed.

A number of media have been used to maintain *N. glaseri*, but these have all been of biological origin and little attempt has been made to develop a defined culture medium. The nematodes grow well on dextrose-agar slants covered with raw kidney, twelve-day mouse embryo extracts or liver; kidney cultures are used routinely. Growth in heat-stable broth is poor, but excellent when raw liver extract (RLE) is added to this medium; raw kidney extracts are not so effective in promoting growth.

These results indicate that RLE contains a factor or factors which considerably enhances growth; this factor is heat-labile. Its chemical nature has not been determined, but on theoretical grounds it is likely that it will prove to be the same as that of the Rb factor essential for the growth of *Coenorhabditis briggsae* and also obtained from liver (p. 428).

There appear to be no unusual cultural conditions necessary for the successful cultivation of *N. glaseri*. Shaking the cultures (at 100  $1\frac{1}{2}$  cm strokes per minute) during incubation results in greater population yields. This result is not unexpected, as a gently rocking medium would have a beneficial effect by assuring the more rapid removal of waste materials and the bringing of fresh nutritional materials into the feeding range of the worm. Cultivation is normally carried out in test tubes at 22° C. Full technical details are given by Stoll (1953, 1959).

### 35.44 Nematodes parasitic in homiothermic vertebrates

#### (a) Intestinal parasites, e.g. *Nippostrongylus muris*

Most of the experimental work carried out on the axenic cultivation of nematodes parasitic in vertebrates has been carried out on strongyles and only a little work has

been attempted with other groups. A substantial degree of success has been achieved, in that development from egg to mature male and female has been accomplished although only infertile eggs have been obtained. In spite of the failure to obtain fertile eggs, this result comprises a considerable achievement, and perhaps the next decade will see the attainment of normal maturation culminating in the production of fertile eggs.

Much of the significant pioneering work in this field has been carried out by Weinstein and Jones (1953-59) to whom reference should be made for the practical details of the various procedures discussed below.

As pointed out earlier (p. 410), many of the species used experimentally have free-living as well as parasitic stages in their life cycles. On this account axenic cultivation of the early larval stages is less difficult in this group of nematodes than in the other parasitic helminth groups, for the physico-chemical conditions of cultivation are less rigid than for those which live in a parasitic habitat. It is not surprising therefore to find that axenic cultivation of the free-living stages of a number of species has been achieved. These species are *Ancylostoma braziliense*, *A. caninum*, *A. duodenale*, *Necator americana*, *Nippostrongylus muris*, *Nematospiroides dubius*, *Haemonchus contortus*. Most of the basic work has been carried out on *N. muris*, the species parasitic in rodents (p. 326), but it is likely that many of the conclusions reached will be applicable to other species.

*Cultivation of free-living stages.* The preparation of eggs in a sterile condition presents little difficulty, as their resistant nature permits the use of such relatively strong antiseptic reagents as 5 per cent antiformin in 10 per cent formalin, 0.1 per cent mercuric chloride or White's mercuric chloride solution. Antibiotic mixtures containing penicillin and streptomycin are used as the final washing solutions. Most workers have also used these antibiotics in their culture media as a precaution against chance contamination.

Glaser and Stoll (1938) carried out the successful cultivation of free-living stages of *Haemonchus contortus* using a complex medium of heat-killed yeast, fresh rabbit kidney, liver extract and agar. It was found, however, that this medium gave only poor yields of filariform larvae when used with *N. muris*. It was shown that both with this species and with dog hookworm larvae, the fresh tissue was the important component and that much higher yields could be obtained using homogenates of chick embryo extract or rat liver. The highest yields were obtained when either CEE<sub>50</sub> or 20 per cent rat-liver extract were used (Weinstein and Jones, 1957). In CEE<sub>50</sub> approximately 75-80 per cent of the larvae reached the filariform stage, but with decreasing concentrations there was a rapid decline in the number developing. High concentrations of embryo extract are thus required for development to proceed. The particulate material present in embryo extract is essential for the development, and when this is removed by

centrifugation or filtration virtually no development to the filariform larvae occurs. The supernatal fluid, however, supports prolonged survival.

It was later found that a medium containing components common to many bacteriological media could support larval development to the filariform stage. This medium, which was purely liquid with no particulate material, contained sodium caseinate, yeast extract and serum. On theoretical grounds, it is likely that similar or related media will be suitable for the cultivation of the free-living stages of the majority of nematode species.

*Cultivation of parasitic stages.* Cultivation of the parasitic stages of nematodes is more difficult on account of the necessity of providing physico-chemical conditions comparable to those in the host habitat and of satisfying the complex requirements of the final maturation stages. In CEE<sub>50</sub>, when the temperature was raised to 37.5° C., filariform larvae of *N. muris* rapidly differentiated to correspond to moderately developed living stages in the rat, but few ever reached even the third moult, although remaining alive and active for many weeks. Unlike the free-living stages, therefore, CEE was nutritionally inadequate for development of the worms to the adult stage.

Eventually it was found that supplementing the extract with rat serum (CEE<sub>70</sub> + 20 per cent rat serum) yielded cultures in which 59 per cent of the worms cultured developed to fifth stage sexually mature adults (Weinstein and Jones, 1959). This is the best result so far achieved with *N. muris*. In these artificially matured worms, some females had about 30 eggs in the uterus and many eggs were deposited in the medium; sperm formation was described as being normal.

Unfortunately, maturation in this medium has been found to be abnormal in two aspects: copulation did not take place, and the eggs produced were infertile although within the normal size range; and maturation *in vitro* was several days longer than *in vivo*.

The latter result is especially significant and is in keeping with the pattern of results obtained in trematodes and cestodes. It suggests that either the nutritional level of the medium is not sufficiently high or the physical conditions of the medium are such that the worm cannot ingest and metabolise it at a sufficiently fast rate. The failure to obtain fertile eggs suggests that special conditions may be necessary for copulation to take place. It will be recalled that a similar situation is found in cestodes where compression of the strobila is necessary to assure insemination (p. 422).

The techniques developed for *N. muris* have been used with some success with a number of other nematodes, and in general the problems encountered and the results obtained have been similar.



(b) *Blood-stream parasites, e.g. Dirofilaria immitis*

*In vitro* cultivation of filariid nematodes has been relatively little investigated. Filariae lend themselves to *in vitro* investigations since they occur in the blood stream, and thus blood or serum from the homologous host provides a suitable maintenance medium; moreover, worms are easily obtained in a sterile condition.

A number of workers have obtained lengthy 'survival' of both microfilariae and adults of a number of species, in serum or saline-serum mixtures. Hawking (1954) kept viable *Litomosoides carni* in Ringer-glucose + 25 per cent horse serum for 14 days. He found, in fact, that microfilariae production was greater *in vitro* (21,600 per day) than *in vivo* (15,000), a result probably attributable to repression of filariid parturition by host mechanisms. It is questionable how much this result represented utilisation of the medium by the parasite and how much represented utilisation of endogenous reserves.

Earl (1959) investigated the behaviour of *Dirofilaria immitis* in various media, using as criteria for 'survival' their ability to acidify the medium and their mobility. Although the former criterion does give some indication of the fact that active metabolism is proceeding, it is a very imprecise criterion unless compared with metabolism *in vivo*. Mobility is likewise an unreliable criterion for an organism may show movement long after its metabolism has ceased to be normal.

Using aerobic conditions and 50 ml of media, he found that the microfilariae of *L. carni* could survive in pure mixture 199 for 4 days, in 199 + 10 per cent horse serum, for 43 days, and in 199 + 30 per cent serum for 63 days. Adult worms were cultured in Eagle's HeLa medium with 10 per cent horse serum and after 10-15 days transferred to pure Eagle's medium at pH 8.0. Females extruded young for 4-7 days and, during the early part of this period at least, the larvae were probably morphologically and physiologically normal, although there is no evidence on this point.

It was also shown that adults die within three days under anaerobic conditions.

Experiments on filariids are clearly only in an exploratory stage of development and not in such an advanced stage as those on strongyles. The experiments described above indicate the general type of results so far obtained. Earl (1959) has summarised the earlier literature.

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# APPENDIX

## ANIMAL PARASITES OF THE LABORATORY RAT AND MOUSE

Classification	Species	Location	Intermediate host
Protozoa: Rhizopoda	<i>Entamoeba muris</i>	caecum	—
	<i>Endolimax nana</i>	caecum	—
Mastigophora	<i>Iodamoeba butschlii</i>	caecum	—
	<i>Trichomonas muris</i>	caecum	—
	<i>Giardia muris</i>	intestine	—
	<i>Hexamita</i> sp.	caecum	—
	<i>Hexamastix</i> sp.	caecum	—
	<i>Chilomastix bettencourti</i>	caecum	—
	<i>Trypanosoma lewisi</i>	blood	—
Ciliata	* <i>Balantidium coli</i>	caecum	—
Sporozoa	<i>Eimeria falciformis</i>	intestine	—
	<i>E. carinii</i>	intestine	—
	<i>E. miyairii</i>	colon, caecum, intestine	—
	<i>E. separata</i>	caecum, colon	—
	<i>Klossiella muris</i>	kidney	—
	<i>Hymenolepis nana</i>	intestine	direct
	<i>H. diminuta</i>	intestine	beetles
Cestoda	* <i>Ochioristica ratti</i>	intestine	beetles
	<i>Hydatigera taeniaeformis (strobilocercus)</i>	liver	—
	<i>Multiceps serialis (coenurus)</i>	muscles	—
	<i>Moniliformis dubius</i>	intestine	beetles
Acanthocephala	<i>Heterakis spumosa</i>	caecum, colon	direct
Nematoda: Oxyuroidea	<i>Aspicularis tetraptera</i>	caecum, colon	direct
	<i>Syphacia obvelata</i>	caecum, colon	direct
	<i>Strongyloides ratti</i>	intestine	direct
	<i>Nippostrongylus muris</i>	intestine	direct
	<i>Trichinella spiralis</i> (adult)	intestine	rat
	<i>T. spiralis</i> (larvae)	muscles	—
	* <i>Trichosomoides crassicauda</i>	bladder	direct
	<i>Trichuris muris</i>	caecum	direct
	<i>Gongylonema neoplasticum</i>	tongue, stomach	beetles
	<i>Mastophurus muris</i>	stomach	beetles

\* Rare but reported.



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